MYCOLOGIA

OFFICIAL ORGAN OF THE MYCOLOGICAL SOCIETY OF AMERICA

Vol. XLII MARCH-APRIL, 1950 No. 2

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PUBLISHED BIMONTHLY FOR

THE NEW YORK BOTANICAL GARDEN

AT PRINCE AND LEMON STS., LANCASTER, PA.

Entered as second-class matter April 20, 1915, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

MYCOLOGIA

Published by

THE NEW YORK BOTANICAL GARDEN

IN COLLABORATION WITH THE

MYCOLOGICAL SOCIETY OF AMERICA

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MYCOLOGIA

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VOL. XLII MARCH–APRIL, 1950 NO. 2

FUNGI IN RELATION TO THE DEGRADATION OF WOOLEN FABRICS[1]

W. LAWRENCE WHITE,[2] G. R. MANDELS,[3] AND R. G. H. SIU[3]

(WITH 7 FIGURES)

INTRODUCTION

The purpose of this investigation was to test the ability of a few common saprophytic molds and a series of human pathogenes to degrade woolen fabrics, and further, to elucidate generally the question of fungous degradation of such materials.

The interest of members of the Quartermaster Laboratories, which had hitherto been primarily in cellulosic materials, was extended, beginning late in 1945, to include studies on the microbiological degradation of woolen fabrics. At that time a report was received from the Office of the Quartermaster General that troops in moving from one location to another were sometimes required to pack woolen clothing hastily into barracks bags, and upon reaching the destination would find the garments molded and in a disagreeable condition. Someone had made a preliminary survey of the molded fabrics and had named *Fusarium moniliforme* Sheld. as the species mainly responsible.

[1] Presented in part at the A.A.A.S. Meetings, Chicago, Dec. 1947.

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[3] Quartermaster General Laboratories.

[MYCOLOGIA for January–February (42: 1–198) was issued February 21, 1950]

The original source of the report and the geographical area in which the annoyance occurred were never learned, and no great amount of faith was placed in the scientific accuracy of the determination of *F. moniliforme* as the causal organism. However, no stretch of the imagination is required to think of such clothing—perhaps damp, dirty, and sweaty—becoming molded when placed in bags in damp climates. The organisms involved, though seriously objectionable, need not necessarily be actual wool degraders.

The microbiological deterioration of woolen fabrics in its fundamental and more purely scientific aspects is part of a broader problem having to do with the nutritional relationship of microorganisms to keratinous materials in general—wool, hair, nails, horn, hoof, feathers, etc. Our knowledge of organisms capable of growing on such materials has been slowly expanded, dating from the observations of *Onygena equina* on old hoofs and horns in the very early days of the history of mycology to the very recent records contained in a series of papers by Dr. John S. Karling. Karling has successfully used various keratinous materials to trap members of the Chytridiales from soil and aquatic habitats. Studies on physiological and chemical aspects of the utilization of keratins by the dermatophytes have recently been reported by Page (**1948**), and a program of fundamental research (Mandels, Stahl & Levinson, **1948**; Stahl, McQue & Siu, **1948**) has been in progress at the Quartermaster Laboratories since shortly after the inception of the work, in 1945, on which the present paper is based.

Turning more specifically to the question of the fungous degradation of woolen fabrics, to which the present paper will be strictly confined, the information available tends to be rather scanty and not very specific. The degradation of "felts" in the paper industry has long been a problem, but the cause presumably is wholly or for the most part bacterial. It is a matter of common observation among those individuals who are engaged in research on deterioration problems that woolen fabrics when subjected to standard soil-burial procedures will decay as readily as do cotton fabrics subjected to similar tests. During the war the number of complaints about the molding of woolen goods was minor compared with the number concerned with the molding of cotton products. To some extent this is due to the fact that in regions where mold problems

are greatest, there is relatively little wool used. On the other hand there is no doubt but that fewer fungi are capable of attacking wool than are capable of attacking cotton. Some cases of molded wool were reported, however, and some samples of such material did reach the writers. There still remains the problem of making detailed studies and tests of some of the fungi that were isolated from such materials.

In 1946 one of the present authors had the opportunity of making a field survey of the degradation of materials around the tropical belt of the world. The more concentrated attention was on cellulosic materials but some incidental observations were also made on the behavior of wool. Samples were returned which exhibited severe degradation by Actinomycetes and at the same time showed resistance to attack by fungi. Caps, field jackets, trousers, etc., made up of combinations of cotton and woolen materials often showed severe molding of the cotton parts, whereas the adjacent woolen portions had remained free from attack. Photographic illustrations of such molded items, accompanied by brief observational data, have already been presented (White, **1946**a, b; White, Siu & Reese, **1948**, p. 628–629).

LITERATURE REVIEW

Those who have worked on the problem of the molding of woolen fabrics from the point of view of the textile industry have not drawn a very clear-cut distinction between molds utilizing finishing materials and those utilizing the substance of the wool itself. It is evident that mold in any case is objectionable. Burgess appears to have been the first worker to enter the field. In a series of papers extending over a ten-year period beginning in 1924 (those concerned with fungi being cited in the bibliography) he dealt primarily with the molding of fabrics in storage and transit. He stressed the role of agents used in the manufacture of woolen goods in rendering the fabric susceptible to molding in storage. These were such materials as soaps, oils, and conditioning fluids. While some substances modified the fiber itself to make parts of it utilizable, it appeared that most of the increased susceptibility was due to the nutritive value of the processing or finishing material. The mildewing properties of both scoured and unscoured wool were

greatly reduced by thorough washing with water. Though the action of heat in rendering wool susceptible to attack was not stressed by Burgess, it had become well known even at that time that wool was subject to modification by the action of heat. Burgess (**1928**) did, however, call attention to the difficulty of testing fungi on wool because of the partial hydrolysis and resulting increased susceptibility brought about by discontinuous steaming which was the method of sterilization that he employed. But, for the most part, those interested in the microbiology of wool gave little consideration to the effect of ordinary laboratory sterilization in rendering wool susceptible to attack by organisms. Humfeld, Elmquist, & Kettering (**1937**) demonstrated fully that standard autoclaving, as well as other heat and various chemical treatments, modified the chemical and physical properties of wool. Their interest was chiefly in sterilization to prevent transmission of human bacterial diseases. Perhaps there was inference that the modification was in the direction of increased susceptibility to bacterial degradation, but no work was done with fungi.

The question of fungi versus bacteria as degraders of woolen goods has been given some attention, with general agreement (Burgess; Thaysen, Bunker, Butlin & Williams, **1939**; Harold, **1941**; Shanor, **1945**; Berk, **1947**) having been reached on certain important phases of the problem, *viz:* that woolen fabrics are ordinarily less susceptible to attack by microorganisms than cotton goods; that wool is none-the-less subject to attack by both fungi and bacteria; that wool is not likely to be attacked unless the humidity is very high, considerably above that necessary for attack on cotton; that any annoyances in damp storage are likely to be due to fungi, a state of wetness or near wetness being necessary for bacterial action.

Several workers have studied the relation of fungous pathogens of the human skin to woolen clothing and have called attention to the possibility of the transmission of skin diseases through woolens. However, cases of actual transmission have been difficult to prove conclusively, and it is still largely presumptive that woolen clothing is more apt to transmit skin diseases than cotton or any other kind of clothing that may be transferred from contact with diseased to normal skin. Gould and Carter (**1931**) inoculated small squares of a sweated woolen athletic jersey with the ringworm fungus, *Tricho-*

phyton interdigitale, and reported that the fungus remained viable for some months, as was also true of inoculum placed on glass, but that it did not grow on the wool. Their conclusion was that pathogenic fungi might live on and be transmitted by woolen clothing but were not likely to grow there. Bonar and Dreyer (**1932**) investigated the growth and transmission of fungi through the medium of clothing, laundries, the wood and debris of dressing room floors, etc. From this work it appears that ringworm fungi may be transmitted through infested skin scales in clothing, either wool or cotton, that they are capable of growth on, and decomposition of, woolen but not cotton fabrics. The temperatures used in standard power laundry practice for white cottons were sufficient to kill the fungi, but that employed for woolens and colored fabrics was doubtful. Dry cleaning solvents had negligible action on the fungi. The fungi tested were *Trichophyton interdigitale, T. rosaceum, Epidermophyton cruris,* and *Microsporon lanosum.* Berberian (**1938**) placed woolen, cotton, and silk stockings in separate Erlenmeyer flasks with a small amount of water, autoclaved them, and inoculated them with spores of *Trichophyton interdigitale.* He reported that at the end of three or four weeks there was marked growth on all of them. He also reported growth on several other materials. Emphasizing the role of footwear in transmission and reinfection he recommended formaldehyde sterilization of such materials. Rogers, Hirschmann and Humfeld (**1940**) found *Trichophyton interdigitale* capable of destroying squares of autoclaved, undyed, laundered, wool blanket material when they were placed on water agar, mineral salts agar or nutrient agars and inoculated with a spore suspension. They emphasized the practical importance of their findings both in the transmission of the athlete's foot disease and in the probable role of the fungus in the utilization of the fabric, thus shortening the period of serviceability. On the basis of the exploratory work reported in the present paper the ringworm fungus *Microsporum gypseum* appeared to be the most suitable of the fungi for biochemical studies on wool degradation. The strain (*PQMD 196*) on which later work was based (Mandels, Stahl, Levinson, **1948**; Stahl, McQue, & Siu, **1948**; Stahl, McQue, Mandels, & Siu, **1948**) was isolated by Mary Downing on September 12, 1946 from a strip of olive-drab woolen cloth that

had been removed July 31 after having been subjected to indoor soil burial at the Philadelphia Quartermaster Depot for one week. It will be noted that strains received later from Dr. Emmons were perhaps equally vigorous.

The role of the common air and soil borne molds in relation to woolen materials is very vague, though several workers have given incidental attention to the identity of organisms isolated from or growing on wool. Burgess (**1924**) emphasized the role of bacteria, stated that it was common knowledge that molds also grow on wool, but obtained mostly negative results when molds were placed on sterile (presumably interval steaming) commercially scoured wool. Eventually he did find a few strains which fruited vigorously —*Aspergillus niger*, an undetermined *Aspergillus* and a *Penicillium*. Among fungi found growing naturally on wool he mentioned *Dematium* (possibly = *Pullularia pullulans*), other Fungi Imperfecti; and yeasts. The same author (**1928**) continuing pure culture tests called attention to the difficulty and uncertainty of such tests due to the hydrolysis caused by the discontinuous steaming. On wool thus treated he obtained growth of *Fusarium* (2 spp.), *Cephalothecium roseum*, *Aspergillus niger*, *A. fumigatus*, *A.* (2 spp.), *Penicillium brevicaule*, *P.* (3 spp.), *Acrostalagmus* sp., and *Trichoderma* sp., but furnished no quantitive data of any kind. Prindle (**1935**) studied the microflora of samples of raw back and shoulder wool from Western U. S. He found large numbers of saprophytic bacteria and molds which he assumed to represent contamination from soil, water, and air. Molds of the genera *Stemphylium*, *Penicillium*, *Oöspora*, *Alternaria*, and *Mucor* were isolated. Most of them were washed away or inactivated in the process of a scouring and drying. Some isolates from all of the foregoing genera except *Mucor* were claimed to cause complete destruction of fiber in worsted yarn, some causing discoloration without destruction. Hirschmann, Zametkin, and Rogers (**1944**) found degreased, steam-sterilized wool to support growth of *Bacillus mesentericus*, *B. subtilis*, and *Actinomyces albus* in the presence of added nutrients. The one fungus with which they worked, *Chaetomium globosum*, was able to grow on wool in the presence of only distilled water. The fiber was relatively resistant to all four

organisms. *C. globosum* was less active in fiber disintegration than *A. albus* which was the strongest of the lot.

Karling, to whom reference was made earlier, has started a series of papers (**1946**; **1947**) on keratinophilic chytrids, indicating that ability to utilize keratin, or possibly even restriction to keratinous materials, may be not uncommon among the Chytridiales. Two species, *Rhizophydium keratinophilum* (**1946**) and *Phlyctorhiza variabilis* (**1947**), both new, were trapped on keratinous substances and found to grow on all common forms of keratin-containing tissues, including wool, but not on other materials. In so far as is known they are strictly soil inhabitants, presumably existing there on bits of keratinous substances, and are of no economic importance in the destruction of fabrics in use or storage.

EXPERIMENTS

Experiment 1. Action of nine common molds on steam sterilized wool serge (table 1).

Nine molds commonly isolated from cotton fabrics after exposure in the tropics, and of known cellulolytic activity, were selected.

Strips of 18-oz., olive-drab, wool serge were prepared by cutting to a size slightly larger than 6 × 1 in. and raveling to exactly that size. These were placed one each in 200 × 25 mm. test tubes each containing 25 ml. of mineral salts (formula A, Greathouse *et al.*, **1942**) medium so that approximately the lower half of each strip was submerged. The tubes were then plugged with cotton and *autoclaved for 20 minutes at 15 lbs. pressure*. Inoculum was made up by agitating sterile distilled water on potato dextrose agar slants on which the fungi were in good sporulating condition. Two ml. of the resulting spore suspension were distributed over the above-liquid portion of each strip by means of a pipette. Incubation of the strips was at 85° F. Following incubation the strips were removed, washed first in 1 : 1000 mercuric chloride, rinsed in water, dried on paper towels in the laboratory atmosphere, subjected to standard conditioning, and broken on the motor-driven Scott tester. Fungal activity was measured in terms of decline in breaking strength.

The results are presented in table 1.

The following points may be made by way of summary and comment.

1. Of 9 organisms tested on wool serge, one, *Aspergillus unguis*, caused a pronounced increase in tensile strength and another, *A. niger*, caused a slight though scarcely significant increase; all others caused a definite decrease, with *Fusarium moniliforme* exhibiting stronger action than the others.

2. The two organisms, which in the foregoing test did not bring about any decline in tensile strength of the wool, had been demonstrated previously by repeated tests not to be cellulose destroyers.

TABLE 1

ACTION OF NINE ORGANISMS ON STEAM STERILIZED WOOL SERGE

Organism[1]	Days incubation	Individual breaks, lbs.	Ave.	Visual growth
None; 2 ml. dist. water	10	54, 53, 53, 55, 54	54	0
	14	57, 50, 56, 56, 55	55	0
Aspergillus unguis[2] *PQMD 8f*	10	70, 67, 67, 70, 64	68	0
	14	70, 70, 69, 71, 73	71	+
Aspergillus niger[2] *PQMD 4j*	10	50, 61, 62, 64, —	59	0
	14	58, 59, 58, 59, 58	58	0
Aspergillus ustus[3] *PQMD 4h*	10	40, 46, 45, 41, 40	42	0
	14	39, 41, 49, 49, 53	46	+
Aspergillus fumigatus *PQMD 6b*	10	38, 38, 38, 48, 42	41	+
	14	34, 28, 31, 34, —	32	+
Fusarium javanicum *PQMD 23d*	10	29, 35, 22, 28, 25	28	++
	14	29, 28, 31, 34, —	31	+++
Stachybotrys atra *PQMD 85m*	10	18, 25, 26, 45, 28	28	+
	14	49, 25, 22, 24, 27	29	+
Torula convoluta *PQMD 4c*	10	25, 19, 19, 19, 19	20	+
	14	18, 10, 16, 10, 19	15	++
Memnoniella echinata *PQMD 1c*	10	15, 19, 15, 17, 15	16	0
	14	18, 16, 19, 16, 18	17	0
Fusarium moniliforme *42 Chem. 70d*	10	10, 10, 13, 14, —	12	+++
	14	12, 14, 15, 12, 11, 15	13	++++

[1] Arranged from top to bottom in order of increasing severity of attack.
[2] Non-cellulolytic.
[3] Of weak cellulolytic ability. All others substantial cellulose destroyers.

Aspergillus ustus, third on the above list, grows well on cotton fabrics with very slow decrease in strength. The six remaining organisms are strong destroyers of cotton fabrics. However, because of the small number of organisms tested on wool, the correlation may be only incidental.

3. Table 1 indicates, in general, little or no decline in strength between the 10th and 14th day of incubation. This is unaccounted for since the two sets of strips appear to have been han-

TABLE 2

ACTION OF FOUR ORGANISMS ON STEAM-STERILIZED WOOL SERGE

Organism	Days incubation	Lbs. breaking strength; av. of 10 replicates	Visual growth
Control, strips autoclaved, incubated, but not inoculated	7	72	0
	9	64	0
	14	61	0
Aspergillus terreus PQMD 82j	7	71	++
	9	62	++
	14	68	+++
Penicillium simplicissimum USDA 15.1	7	71	+
	9	65	+
	14	69	++
Pestolotia sp. Panama 57B5G	7	69	+
	9	61	++
	14	67	++
Trichoderma viride PQMD 18e	7	64	+
	9	57	++
	14	57	+++

dled correctly, including breaking by the same individual on the same machine.

Experiment 2. Activity of four miscellaneous fungi on steam-sterilized wool serge (table 2).

The materials and methods were the same as in the previous set of tests. The organisms tested, the periods of incubation, and the results obtained are indicated in table 2.

The following notes were recorded concerning the growth characteristics of the organisms on the wool.

PQMD 82j. Fine white hyphal fuzz developing over whole surface of strip by 7th day and showing a certain amount of scattered pale brown sporulation.

USDA 15.1. Scant white mycelial growth developing slowly with some sporulation at extreme upper end of strip.

Panama 57B5G. White cottony mycelium developing on lower part of exposed portion of strip; no noteworthy increase in growth after 9th day; no sporulation.

PQMD 18e. At 7 days showing a fine fuzzy white mycelial growth more or less evenly distributed over the above-water portion of the strip and a few yellowish fruiting pustules appearing at margins and top; at 9 and 14 days showing considerable white mycelium with yellowish green fruiting hammocks scattered over the cloth.

It is evident from the table and the foregoing paragraphs that although there was enough carbon present, probably as extraneous matter, to enable all of these fungi to make a certain amount of growth, they were nevertheless incapable of attacking the wool directly. It may be noted, incidentally, that all four of these fungi have been shown to break down cotton fabrics under similar test procedures.

Experiment 3. Activity of twelve miscellaneous organisms (including those tested on sterilized wool in experiment 1) on unsterilized wool challie (table 3).

Strips of gray wool challie, raveled to exactly 6 × 1 inches, were wet out in 0.05 per cent Gardinol WA, rinsed in distilled water and placed 1 each in test tubes of 200 × 25 mm. size, each containing 25 ml. of sterile (Greathouse formula A) mineral salts solution. This amount covered a little less than one-half of the strip.

The inoculum was prepared as follows: Each organism to be tested was streaked on a potato dextrose agar slant in a standard 20 mm. test tube and incubated at room temperature for about 3 weeks to obtain good sporulation. To each tube was added approximately 10 ml. of the sterile mineral salts solution.

The spores were loosened and brought into suspension by agitation with a sterile pipette, then the suspension was poured into a

TABLE 3

ACTION OF TWELVE MISCELLANEOUS FUNGI ON UNSTERILIZED WOOL CHALLIE

Organism	Days incubation	Breaking strength, lbs.	Amount of visual growth at end of test
Uninoculated control	6	34	0
	9	23	0
	12	17	0
Aspergillus fumigatus PQMD 6b	6	32	0
	9	27	0
	12	21	0
Aspergillus niger PQMD 45d	6	36	0
	9	27	0
	12	21	0
Aspergillus unguis PQMD 8f	6	39	0
	9	14	0
	12	11	0
Aspergillus ustus PQMD 4h	6	37	+
	9	27	+
	12	22	+
Fusarium javanicus PQMD 23d	6	31	0
	9	21	0
	12	11	0
Fusarium moniliforme 42 Chem. 70d	6	37	0
	9	33	0
	12	22	0
Fusarium sp. PQMD 197	6	32	0
	9	19	0
	12	9	0
Gliomastix convoluta PQMD 4c	6	33	0
	9	16	0
	12	18	0
Microsporum gypseum PQMD 196	6	0	+
	9	0	++
	12	0	++
Stachybotrys atra PQMD 85m	6	32	0
	9	25	0
	12	18	0
Actinomycete PQMD 195	6	35	+
	9	32	+
	12	16	+
Memnoniella echinata PQMD 1c	6	33	0
	9	27	0
	12	18	0

large sterile test tube containing enough sterile mineral salts solution to make the total inoculum up to 35 ml. Two ml. of the suspension were then pipetted evenly over the upper portion of the strip, *i.e.*, the portion which was not submerged in the nutrient solution.

The results are presented in table 3.

In all cases there was a putrid odor and cloudiness in the liquid by the period of the first harvest. In all except *Microsporum gypseum* the results were much the same, *i.e.*, there was steady and pronounced decrease in the tensile strength of the wool strips accompanied by no noteworthy growth of the fungi. There were no noteworthy differences between the sets that were used as controls and those inoculated. *Microsporum gypseum* was in a category by itself with respect to rapidity of action and amount of visual growth produced. It is assumed that in all instances except the *M. gypseum* inoculation, the decline in strength was due to the action of bacterial contaminants. In conclusion, it may be said that under the conditions of this set of tests, wool, which had not been modified by steam heating, proved resistant to degradation by ten common molds and an Actinomycete but was decomposed readily by *Microsporum gypseum*.

Experiment 4. Action of twelve miscellaneous mold fungi on autoclaved as compared with non-autoclaved wool charmeen (table 4).

Strips of gray wool charmeen were raveled to exactly 3 × 1 inches in size. They were dipped in 50 per cent ethyl alcohol, then washed 4 times in distilled water. This was mainly for the purpose of wetting them out. Three variations were introduced into the experiment as will be described further on. Petri dishes of standard 90 mm. size were prepared, each containing a layer of glass beads and 10 ml. of a mineral salts solution. The wool strips were placed two, side-by-side, in each dish. The amount of solution used was sufficient to keep the cloth moist but not submerged. It had the following composition: NH_4NO_3, 3 g.; $MgSO_4 \cdot 7H_2O$, 2.22 g.; KH_2PO_4, 2.59 g.; K_2HPO_4, 2.21 g.; distilled water, 1000 ml.; pH 6.5. With the exception of the controls, 1 ml. of a spore

TABLE 4

ACTION OF TWELVE MISCELLANEOUS MOLDS ON NON-AUTOCLAVED (A) AS COMPARED WITH AUTOCLAVED WOOL CHARMEEN WITH (C) AND WITHOUT (B) YEAST EXTRACT

Organism	Treatment	Tensile strength retained				pH		Observation after 9 days growth
		5 days		9 days		5 days	9 days	
		Lbs.	%*	Lbs.	%*			
Control—not inoculated	A	58		56		6.3	6.45	None
	B	40		33		6.3	6.4	None
	C	39		39		6.4	6.4	None
Alternaria tenuis PQMD 26a	A	57	98	58	104	6.4	6.5	Very slight
	B	19.3	49	14	36	7.7	7.7	Moderate; scanty sporulation; purpling of wool
	C	16.1	41	14	36	7.7	7.8	Moderate mycelium and sporulation; deep purpling
Aspergillus fumigatus PQMD 45h	A	55	95	53	95	6.5	6.8	Slight growth and sporulation
	B	26	67	20	51	7.9	7.75	Heavy sporulation
	C	23	59	22	56	7.85	7.75	Very heavy sporulation
Chaetomium funiculum PQMD 33c	A	54	93	53	95	6.35	6.5	Very slight indication of growth
	B	34	87	31	79	7.0	7.6	Moderate perithecial production
	C	31	79	33	85	7.3	7.5	Heavy perithecial production
Chaetomium globosum PQMD 79g	A	54	93	51	91	6.45	6.6	Slight indication of growth
	B	28	72	23	59	7.7	8.0	Moderate growth and perithecial formation
	C	26	67	23	59	7.8	7.9	Heavy mycelium; moderate perithecial formation
Cladosporium herbarum PQMD 17b	A	57	98	55	98	6.4	6.4	Slight growth
	B	37	95	35	90	6.5	6.9	Moderate growth with darkening of cloth
	C	38	97	35	90	6.5	6.75	Moderate sporulation

* Note: in calculating the % retention in tensile strength after growth on the autoclaved strips, the figure 39 lbs. was used; for non-autoclaved strips (A) 58 lbs. was used. The figure of 33 lbs. for the 9-day harvest of the control for series B is considered to be in error, possibly due to unobserved bacterial contamination.

TABLE 4—*Continued*

Organism	Treatment	Tensile strength retained				pH		Observation after 9 days growth
		5 days		9 days		5 days	9 days	
		Lbs.	%*	Lbs.	%*			
Fusarium moniliforme Chem. 70d	A	56	96	52	93	6.5	6.65	None
	B	11	28	6	15	8.15	8.5	White cottony to slimy growth; cloth brownish
	C	14	36	7	18	8.25	8.5	Heavier growth; cloth brownish
Gliomastix convoluta PQMD 4c	A	57	98	57	102	6.4	6.5	Very slight indication of growth
	B	10	26	6	15	7.8	8.0	Moderate growth and sporulation; tan discoloration
	C	13	33	5	13	7.75	7.9	Moderate growth and sporulation; tan discoloration
Memnoniella echinata PQMD 1c	A	58	100	57	102	6.4	6.35	Very slight growth
	B	34	87	19	49	7.15	7.5	Very slight growth; brownish discoloration
	C	22	56	14	36	7.4	7.8	Very slight growth; deeper discoloration
Metarrhizium Anisoplae NRRL 1945	A	57	98	54	96	6.4	6.4	Very slight growth
	B	29	74	19	49	7.25	7.9	Slight to moderate mycelium; scanty sporulation
	C	28	72	21	54	7.25	7.9	Slight to moderate mycelium; scanty sporulation
Metarrhizium brunneum NRRL 1944	A	56	96	53	95	6.35	6.35	Very slight growth
	B	34	87	22	56	6.6	7.5	Moderate growth and sporulation
	C	29	74	19	49	6.8	7.1	Moderate growth and fairly heavy sporulation
Microsporum gypsum PQMD 196	A	18.2	31	10	18	7.9	8.1	Moderate white mycelial mat covering cloth
	B	—	—	5	13	8.4	8.3	Heavy white mycelial mat
	C	—	—	7	18	8.4	8.25	Very heavy white mycelial mat
Myrothecium verrucaria PQMD 185	A	43.5	75	43	77	7.2	6.9	Slight mycelium and sporulation
	B	19	49	20	51	7.7	8.0	Moderate mycelium; some sporulation
	C	19	49	18	46	7.7	8.1	Moderate mycelium; some sporulation

suspension was distributed by pipette on each strip. The inoculum in each case was prepared from a 19-day-old culture of the organism on potato dextrose agar in a 250 ml. Erlenmeyer flask. Sterile distilled water was used to make the spore suspensions. The dishes were incubated in a high humidity chamber at about 85° F.

For each organism tested 30 strips of wool were used. These were divided into 3 lots of 10 each as follows: (A) strips not autoclaved, (B) strips subjected, after wetting them, to standard autoclaving of 15 minutes at 15 pounds pressure prior to transferring under sterile conditions to the petri dishes, (C) strips autoclaved and yeast extract (0.01 per cent) added to the mineral salts medium on which they were incubated.

Following incubation, the strips were removed from the dishes, conditioned, and broken on the Scott tester as in the previous experiments.

The results are presented in table 4. While all of the fungi tested attacked the autoclaved wool, only two, *Microsporum gypsum* and *Myrothecium verrucaria*, degraded the non-autoclaved wool. Addition of yeast extract was without significant effect except for *Memnoniella echinata* in which case the degradation was accelerated slightly. In all cases where the wool was attacked, the pH increased, the increase in pH being a function of the extent of degradation.

Experiment 5. Action of three fungi on autoclaved wool (table 5).

Results of another experiment carried out the same as experiment 4, but employing only autoclaved wool without yeast extract being added, are shown in table 5. Although *Oöspora lactis*, a strongly proteolytic organism, had no effect, the *Penicillium* and *Syncephalastrum racemosum* degraded the wool slowly.

Experiment 6. Effect of Myrothecium verrucaria on autoclaved and non-autoclaved wool cloth (table 6).

A previous experiment (number 4) had indicated that *Myrothecium verrucaria* could degrade non-autoclaved wool to a slight extent. It was thought that complete degradation of the wool may have been prevented by the high pH of the culture solution after

growth had occurred, or from the accumulation of inhibitory waste products. To test this hypothesis a series of cultures was set up employing the same methods as those of experiment 4. Harvests were taken at 3, 6, 10 and 12 days after inoculation (*M. verrucaria, USDA 1334.2*) at which times the culture solution was removed by tipping the Petri dishes and sucking off the liquid with a glass tube connected to an aspirator. Ten ml. of fresh nutrient solution (see experiment 4) were added to each remaining culture. Determinations of pH, using a glass electrode, were made by combining the solutions from the three Petri dishes harvested for each

TABLE 5

ACTION OF THREE FUNGI ON AUTOCLAVED WOOL

Organism	Breaking strength retained				pH		Notes
	5 days		8 days		5 days	8 days	
	lbs.	Ret'n	lbs.	Ret'n			
Oospora lactis JQMD 433	42	104	38	94	6.45	6.5	No evidence of growth or germination
Penicillium sp. *PQMD 229*	39	96	35	86	6.6	6.6	Some sporulation where chunks of inoculum.
Syncephalastrum racemosum PQMD 57a	39	96	34	84	6.5	6.7	Sparse mycelium; abundant sporangia
Control—not inoculated	40	100	41	100	6.4	6.4	

of the two treatments at each harvest. It is evident from the results (table 6) that the limited degradation of wool, resulting from the action of this fungus, is not due to the creation of an unfavorable reaction in the culture solution. The data leave some questions regarding the role of inhibitory waste products since it is possible that the solution held in the cloth strips by capillary action may have contained sufficient amounts to account for the results. It is not believed, however, that the observed limited degradation of the wool is due to this cause. Some other mechanism must obtain. In the processing of wool the fibers are subjected to brief exposure

to heat, alkali and acid. It is quite conceivable that the slight modification of the wool which undoubtedly results from such treatment enables *Myrothecium verrucaria* to grow and that this organism is incapable of attacking undamaged keratin.

Myrothecium verrucaria is commonly isolated from the soil or is found sporulating on plant materials. It is, of course, well known as a strong degrader of cellulose under laboratory conditions. However, there are in the Herbarium of the U. S. Department of Agriculture at Beltsville two specimens which show it inhabiting and sporulating on old cow horn. Both collections are from

TABLE 6

EFFECT OF *Myrothecium verrucaria* ON AUTOCLAVED AND NON-AUTOCLAVED WOOL CLOTH

Time, days	Autoclaved					Non-autoclaved				
	pH		Tensile strength* retained, lbs.		% retention Inoculated	pH		Tensile strength* retained, lbs.		% retention Inoculated
	Inoc.	Control	Inoc.	Cont.		Inoc.	Control	Inoc.	Cont.	
3	7.4	6.65	30	38	74	6.7	6.55	49	57	86
6	7.6	6.55	23	42	57	6.65	6.5	44	57	77
10	7.35	6.4	16	41	39.5	6.5	6.35	40	57	70
12	6.75	6.35	14.5	—	36	6.25	6.2	39.5	57	69

* Average value of 40 lbs. used for calculating % retention.

Louisiana, one taken in 1888 and the other in 1890. One appears under the label *M. roridum* and the other as *M.* sp.

Experiment 7. Action of medical fungi and related forms on autoclaved and non-autoclaved wool charmeen (FIG. 1).

Strips of wool cloth (a gray wool charmeen, 70's grade), $\frac{3}{4}'' \times 4''$, were wetted in 50% ethanol, rinsed 4 times in distilled water and placed in 25 × 200 mm. Pyrex test tubes. Half of the tubes were autoclaved (15 lbs. for 15 min.); the remaining tubes were not sterilized. Twenty-five ml. of sterile nutrient solution (see experiment 4) were added aseptically to the tubes. This volume of solution was sufficient to cover about one-half the length of the wool strips. The strips were inoculated in triplicate by drawing

a loopful of inoculum upward from the surface of the solution to the top of the strip. Spore suspensions for the inoculum had been prepared by adding sterile distilled water to cultures grown in

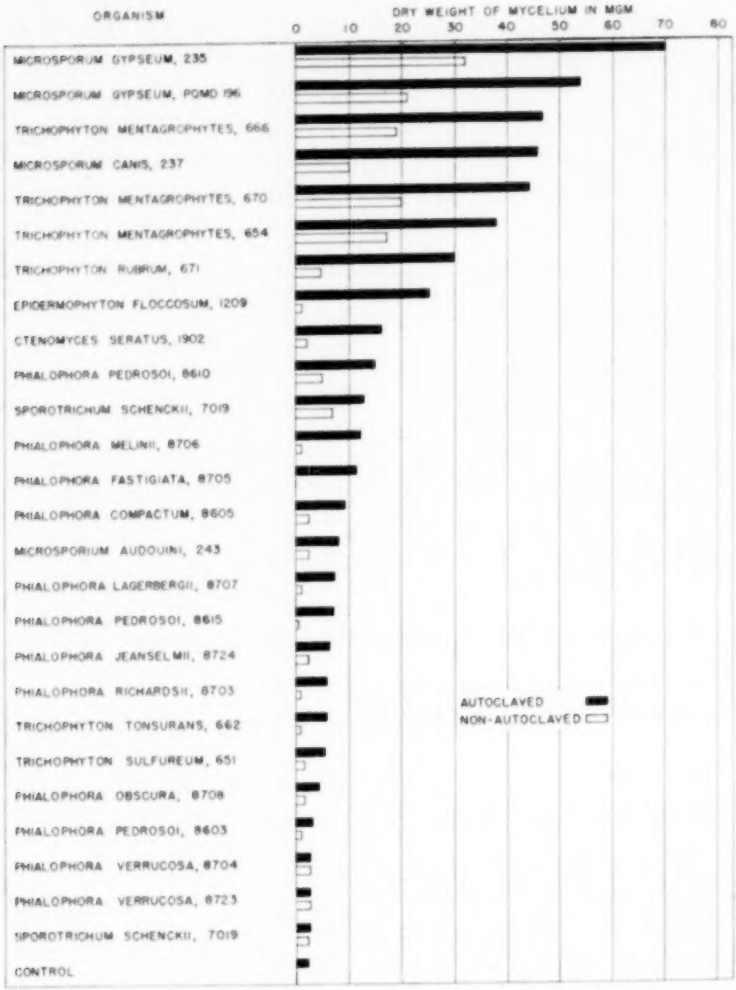


FIG. 1. Growth of dermatophytes and similar or associated fungi on autoclaved and non-autoclaved wool charmeen. The numbers following the names of the organisms, with the exception of the second from the top, are those under which the cultures were received from Dr. Chester W. Emmons of the National Institute of Health.

test tubes on potato dextrose agar for 6 weeks at about 30° C. At the end of the incubation period the Petri dishes and their contents were subjected to autoclaving to kill the organism. The nutrient solution was then decanted off and the wool plus mycelium washed with 0.1 N HCl and distilled water to remove any phosphate precipitate. The contents of the three replicate tubes were then combined, the excess water decanted off and about 50 ml. of boiling 10% NaOH added to dissolve the wool. After cooling, about 60 ml. of distilled water were added and the mycelial residue was filtered off in previously weighed, fritted glass crucibles of medium porosity. The crucibles were then dried overnight at 105° C. and weighed.

The results are presented in figure 1.

The following conclusions may be drawn:

1. Growth of the fungi used varied from luxuriant to little or no growth on both autoclaved and non-autoclaved wool. The dry weight data show similar continuous variation. Thus the differences between the various isolates used are entirely of a quantitative nature, no sharp qualitative differences being observed.

2. Growth on autoclaved wool was greater than that on non-autoclaved wool in all cases where significant growth occurred. No constant relation was found, however, between the two values.

3. With respect to the keratinolytic "activity" of the isolates tested, only certain members of the genera *Microsporum* and *Trichophyton* showed significant ability to degrade wool.

CTENOMYCES-LIKE FUNGI ON WOOLEN FABRICS

Worthy of brief comment at this point is a group of seven isolates having the general morphology of the conidial stage of *Ctenomyces*. All except one of these were obtained from woolen fabrics.

Preliminary work suggests that on the basis of gross colony characteristics correlated with the shape of the conidia the cultures may be segregated into three groups, viz.:

Group A. (FIGS. 2–4)

JQMD 1087. Isolated 14 July 1945 from a woolen overcoat from Guadalcanal.

JQMD 1277. Isolated 21 February 1946 from a woolen overcoat from Guadalcanal (FIG. 2).

PQMD 199. Isolated 5 July 1946 as a contaminant on a strip of gray wool challie that had been inoculated with another organism (FIG. 3).

PQMD 208. Isolated 22 November 1946 as contaminant on strip of gray wool challie that had been inoculated with another organism (FIG. 4).

Group B. (FIG. 5)

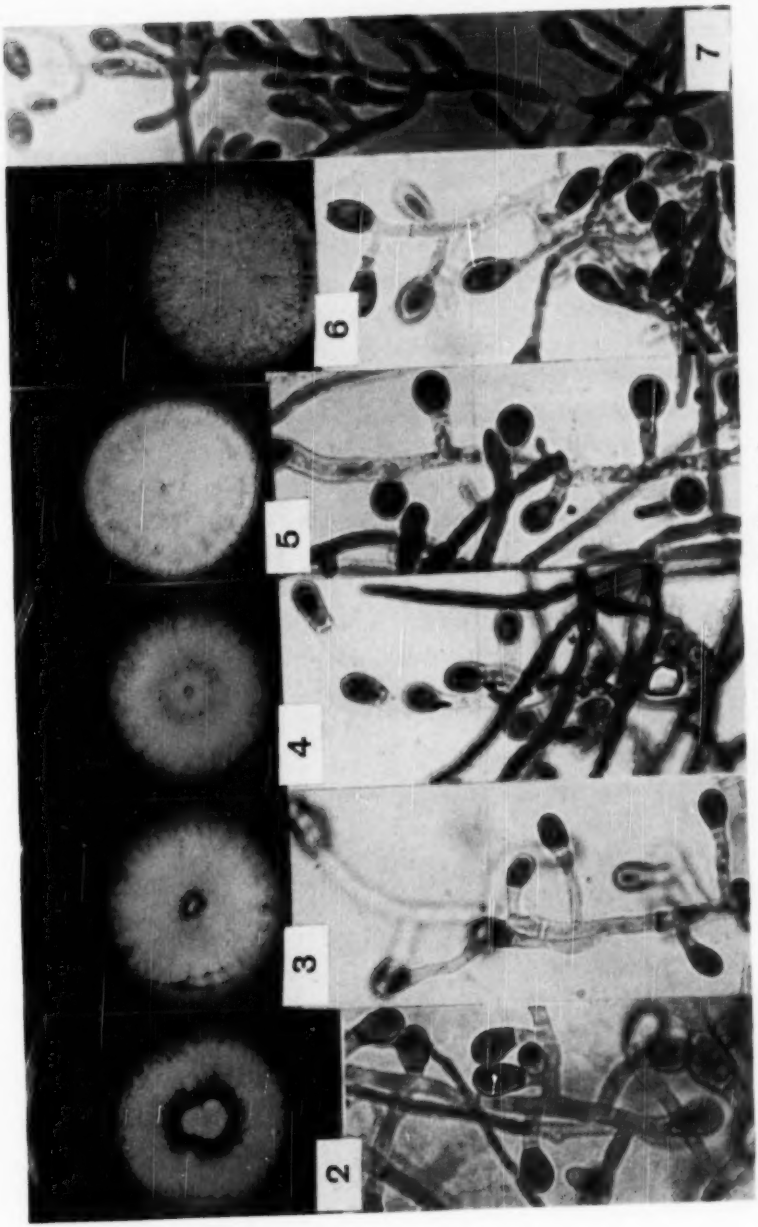
PQMD 287. Isolated from deteriorated wool serge exposed experimentally in a "tropical" room at the British Ordnance Laboratory, Cawnpore, India; culture and sample received at Philadelphia in May 1947.

Group C. (FIGS. 6–7)

JQMD 1070. Isolated 26 June 1945 from a sample of deteriorated tent top from Oro Bay, New Guinea (FIG. 7).

JQMD 1282. Isolated 1 March 1946 from wool serge from Guadalcanal (FIG. 6).

Whether the cultures represent one species or three cannot be determined from the information now available. The photographs in figures 2–6 are of the organisms grown in parallel series for 18 days under room conditions on Bacto Difco potato dextrose agar, 2% dextrose, 35 cc. of the medium in standard 90 mm. Petri dishes. The colonies are white and show about the same growth rate. It is obvious from the figures (2–4) that *JQMD 1277*, *PQMD 199*, and *PQMD 208* represent one species. The colonies have thin margins and show a tendency for the aerial mycelium of the central areas to sink and become water-soaked. The conidia are obovoid with obtuse, rounded apices. *PQMD 287* (FIG. 5) shows a heavier growth, abrupt margin, no water-soaked appearance in center. The conidia are more broadly obovoid, nearly globose. In *JQMD 1282* (FIG. 6) the colony is striate, and of wholly different texture from the others. The conidia tend to be relatively elongate with the apices pointed. The photomicrograph of *JQMD 1070* (FIG. 7) was taken in a different laboratory from the others and probably



FIGS. 2–7. *Ctenomyces*-like fungi.

under another set of conditions of culture and staining, but it does show clearly the elongate spores corresponding with those of figure 6.

The evidence that these fungi are of significance in wool deterioration is more or less circumstantial. All of the cultures were isolated from wool except one. In the case of those originating at the Jeffersonville Laboratory (*JQMD*) the fabric from which they came was not seen by the writers. In the *PQMD* series, however, the organism was, in each instance, found growing and sporulating on the material. *PQMD 199* appeared on a strip of gray wool challie that had been immersed in Stoddard Solvent for 10 minutes, was then placed on glass beads in contact with a mineral salts solution, and inoculated with another organism that failed to make appreciable growth. In a preliminary test employing autoclaved gray wool challie it caused a more rapid decline in tensile strength than did *Microsporium gypseum, PQMD 196*. Culture *PQMD 208* was obtained under similar conditions to those of *199* but was on several strips of unsterilized and uninoculated experimental control material. Culture *PQMD 287* was received for identification from the British Ordnance Laboratory, Cawnpore, India (their number D–381), accompanied by a sample of olive drab woolen fabric from which it had been isolated and on which it was present in good sporulating condition. It was indicated as having come from a wool serge coat exposed in a "tropical chamber" for test purposes. The letter of communication (from Dr. T. S. Subramanian) referred to it as a wool destroying fungus capable of disintegrating wool fiber completely in the course of a month.

The suggestion that these fungi might have perfect stages in *Ctenomyces* was made by Dr. C. W. Emmons who examined cultures *PQMD 199* and *287*. They have been discussed here because as conidial stages of *Ctenomyces* or some closely related Gymnoascaceous genus they tie in with such genera as *Microsporum* and *Trichophyton* which are also of presumed Gymnoascaceous derivation. It is among such forms as these, as distinct from the great array of common saprophytic molds, that the real degraders of unmodified wool are to be found.

CONCLUSIONS

1. As woolen fabrics are placed on the market—perhaps with the fiber slightly but not seriously modified by prior processing—they are less subject to fungous attack in storage and use than are cotton fabrics.

2. The species of fungi which attack woolen textiles are relatively very few as compared with the range of forms which attack cotton materials.

3. A certain few species of fungi are capable of degrading woolens with a rapidity comparable to the action of the stronger cellulose-destroyers against cotton materials.

4. The fungi that are capable of such activity are commonly found in the conidial stage only, are of similar morphology, belong among, or are closely allied with, the ringworm or skin disease fungi known as dermatophytes, and are probably of Gymnoascaceous derivation.

5. Despite certain reports that are seemingly to the contrary, laboratory tests and field observations by the present writers indicate that the common molds of industrial and military materials do not, as a rule at least, attack wool.

6. Woolen fabrics are modified by standard steam sterilization (autoclaving) in such a way as to render them, in laboratory tests, subject to attack by a wide range of common molds.

7. There is no clear qualitative distinction between those fungi which degrade either or both modified and non-modified wool and those which do not.

8. Degradation is accompanied by an increase in pH, the increase being roughly proportional to the extent of degradation.

9. Woolen fabrics when subjected to the common soil burial tests, where bacterial action is probably of major importance, decay as readily as cotton fabrics subjected to similar conditions.

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DESCRIPTION OF FIGURES

FIGS. 2–7. Ctenomyces-like fungi. 2, *JQMD 1277;* 3, *PQMD 199;* 4, *PQMD 208;* 5, *PQMD 287;* 6, *JQMD 1282;* 7, *JQMD 1070.* All except 7 grown on Bacto Difco potato dextrose agar (2% dextrose) under room conditions for 18 days, the microscopic stages mounted in KOH-phloxine; growth conditions and mounting medium for 7 unknown. Colonies × approx. ½, photomicrographs × 1000. (Colony photos by Photographic Dep't, Phila. Q. M. Depot; photomicrographs 2–6 by Mary Downing, Phila. Q. M. Depot; figure 7 courtesy Biol. Lab., Jeffersonville Q. M. Depot.) For discussion see text.

SOME NOTEWORTHY RUSTS. III *

M. J. Thirumalachar

(with 8 figures)

Hapalophragmium mysorense sp. nov.

Pycnia amphigena, subcuticularia, conoidea, applanata, absque ostiolaribus, paraphysibus, evoluta super infectionis maculas hypertrophitas, 2–10 mm. diam. Aecia uredinoidea, amphigena, evoluta, circum pycnia, erumpentia atque pulverulenta, paraphysata; aeciosporae similes urediosporis, 24–28 × 20–24 μ.

Uredia subepidermalia, hypophylla, erumpentia, paraphysata; paraphyses peripherales, tenuiter incurvatae, atque extus projectae, 54–70 × 8–9 μ; urediosporae pedicellatae, evolutae singillatim, luteo-brunneae, ovato-oblongae, 24–28 × 20–24 μ; parietes 1.5 μ crassi, ornati 2–3 germinationis poris equatorialibus, dense echinulati. Telia absque urediis; teliosporae pedicellatae, 3-cellulatae, luteo-brunneae, cellula impari terminali; apice obconico atque protrudenti, ornato germinationis poro in singulis cellulis, 39–54 × 28–40 μ, parietes laeves, sed minutissimis tuberculis ornati prope germinationis porum; pediculis hyalinus deciduus.

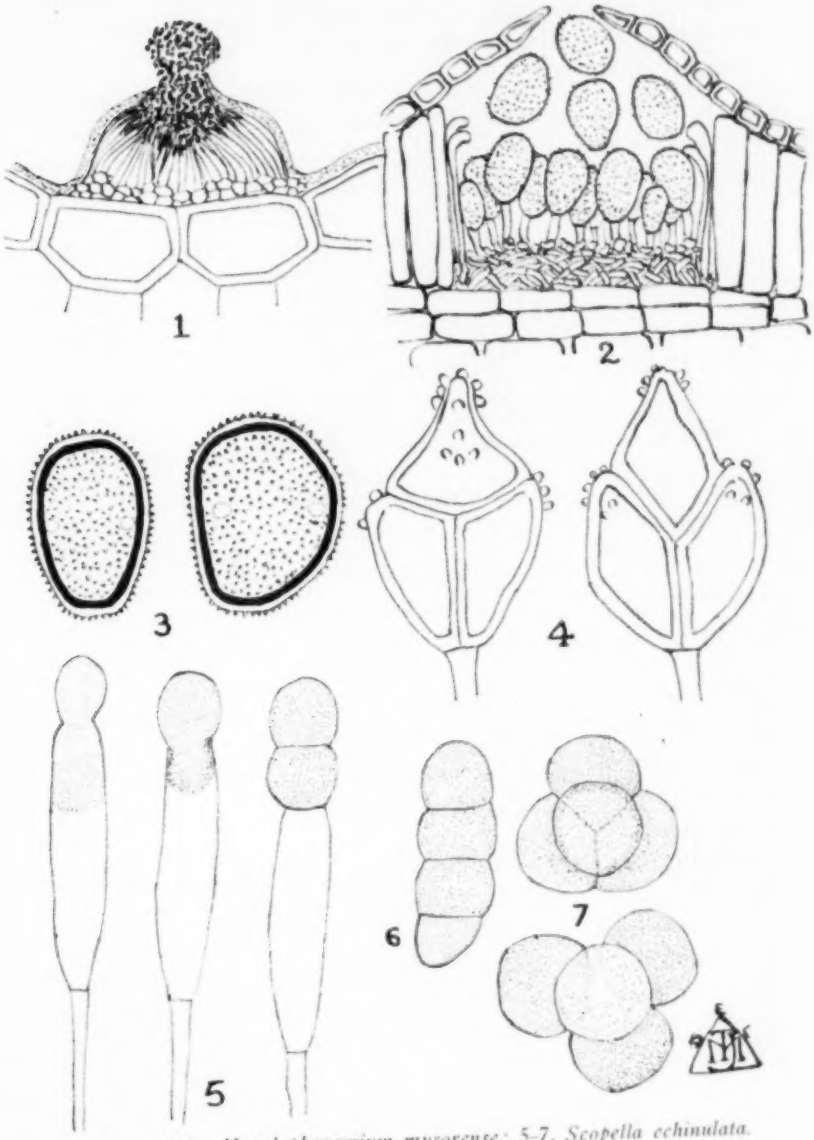
In foliis *Derris Benthamii*.

Pycnia amphigenous, subcuticular, conoid, applanate, without conspicuous ostiolar paraphyses, developed on hypertrophied infection spots, 2–10 mm. in diameter. Aecia uredinoid, amphigenous, developed surrounding the pycnia, subepidermal, erumpent and pulverulent, paraphysate; aeciospores resembling the urediospores, 24–28 × 20–24 μ.

Uredia subepidermal, hypophyllous, erumpent, paraphysate; paraphyses peripheral, slightly incurved, and projecting out, 54–70 × 8–9 μ; urediospores pedicellate, developed singly, yellowish brown, ovate-oblong, 24–28 × 20–24 μ, wall up to 1.5 μ thick, with 2 to 3 equatorial germ pores, closely echinulate. Telia developed within uredia, teliospores pedicellate, three-celled, yellowish brown, odd cell terminal, apex obconical and protruding, with an apical germ pore in each cell, measuring 34–54 × 28–40 μ; wall smooth but with warts at the region of the germ pore; pedicel hyaline, deciduous.

Hab. on leaves of *Derris Benthamii* Thw., Kemmangundi, Mysore, 27–4–1949, leg M. J. Thirumalachar (**Type**), type deposited

* Part I published in Mycologia **37**: 295–310, 1945; Part II, Mycologia **39**: 231–248, 1947.



FIGS. 1–4. *Hapalophragmium mysorense*; 5–7, *Scopella echinulata*.

in Arthur Herbarium, Purdue University, Lafayette, Indiana, U. S. A.; in Herb. C. M. I. Kew, England, and in Herb. Crypt. Ind. Orient., New Delhi, India (FIGS. 1–4).

There are four other species of *Hapalophragmium* recorded on species of *Derris* with which the present species has to be compared. All the four known species are closely related to each other and show overlapping of characters. Their important characters are summarized below:

Species	Host	Uredio-spore	Telio-spore	Wart in the germ pore of teliospores
H. Derridis Syd.	*D. uliginosa*	27–36 × 19–25 μ	38–58 × 27–38 μ	Not known
H. annamalaiensis Ramakrishnan T.S. & K.	*D. eualata*	24–38 × 19–27 μ	35–58 × 17–34 μ	Absent
H. setulosum Pat.	*D.* sp.	18–20 × 15–18 μ	35–44 × 32–36 μ	Present
H. pulchrum (Racib.) Syd.	*D. elliptica*	20–22 × 18–20 μ	36–42 × 40 μ	Present
H. mysorense Thirum.	*D. Benthamii*	24–28 × 20–24 μ	39–54 × 28–40 μ	Present

It is apparent that the species of *Hapalophragmium* on *Derris* are closely related and show intergrading of characters. *H. mysorense* differs from *H. setulosum* and *H. pulchrum* (which also possess warts near the germ pores) in the size of the uredio- and teliospores.

Subcuticular pycnia and subepidermal uredinoid aecia have been observed in *H. mysorense*. *H. Derridis* Syd. which is the type of the genus *Hapalophragmium*, founded by the Sydows (**1901**), has been pointed out to show close relationship to other species of *Hapalophragmium* on *Derris* including *H. mysorense*. The finding of the pycnial and aecial stage for *H. mysorense* may be taken as representing the condition present in the genus *Hapalophragmium*.

The gall-forming rust *Hapalophragmium ponderosum* has been described by Sydow & Butler (**1912**) from India. Only the telial stage was first described, but later Thirumalachar (**1941**) noted the occurrence of subepidermal pycnia for the rust. This is in

contrast to the subcuticular condition just described for *Hapalophragmium* and constitutes a different type of sorus structure. The rust therefore belongs to a separate but closely related genus, and the writer proposes the name *Hapalophragmiopsis* for its accommodation.

Hapalophragmiopsis gen. nov.

Pycnia subepidermalia, conoidea, absque conspicuis paraphysibus ostiolaribus. Aecia atque uredia incognita. Telia subepidermalia, erumpentia, aparaphysata; teliosporae pedicellatae, 3-cellulatae, spora impari terminali ut in Hapalophragmio, luteo-brunneae, singulis teliosporae unico germinationis poro ornatis; pediculus hyalinus et deciduus.

Species typica: *Hapalophragmiopsis ponderosum* (Syd. & Butler) Thirumalachar (FIG. 8, A to D).

Pycnia subepidermal, conoid, without conspicuous ostiolar paraphyses. Aecia and uredia unknown. Telia subepidermal, erumpent, aparaphysate; teliospores pedicellate, 3-celled, odd spore terminal as in *Hapalophragmium*, yellowish brown, with a single germ pore in each cell of the teliospore; pedicel hyaline, deciduous.

Type species: *Hapalophragmiopsis ponderosum* (Syd. & Butler) Thirumalachar on *Acacia leucophloea* Willd.

H. ponderosum causes the production of large gall formations and imparts a striking appearance to *Acacia leucophloea*, a medium-sized tree in India (FIG. 8, A). The galls are produced as a result of infection of the peduncles of the inflorescence and also young fruits. An account of the teliospore germination and pathological histology of the gall was given by Thirumalachar (**1941**). *H. Acaciae* Baccarini is also a gall-forming rust on *Acacia* sp. in Somaliland, Africa. It is reported to resemble *H. ponderosum* closely. This rust may prove to be a species of *Hapalophragmiopsis* when its pycnial stage is discovered.

ON SCOPELLA ECHINULATA (NIESSL) MAINS.

The genus *Scopella* was established by Mains (**1939**) based on *S. echinulata* parasitic on *Bassia latifolia* in India. Subcuticular pycnia, and subepidermal uredia and telia were described, the teliospores being pedicellate, thin-walled and germinating immediately at maturity. The clustering of the urediospores and teliospores on laterally free sporogenous basal cells was noticed by Mains, who

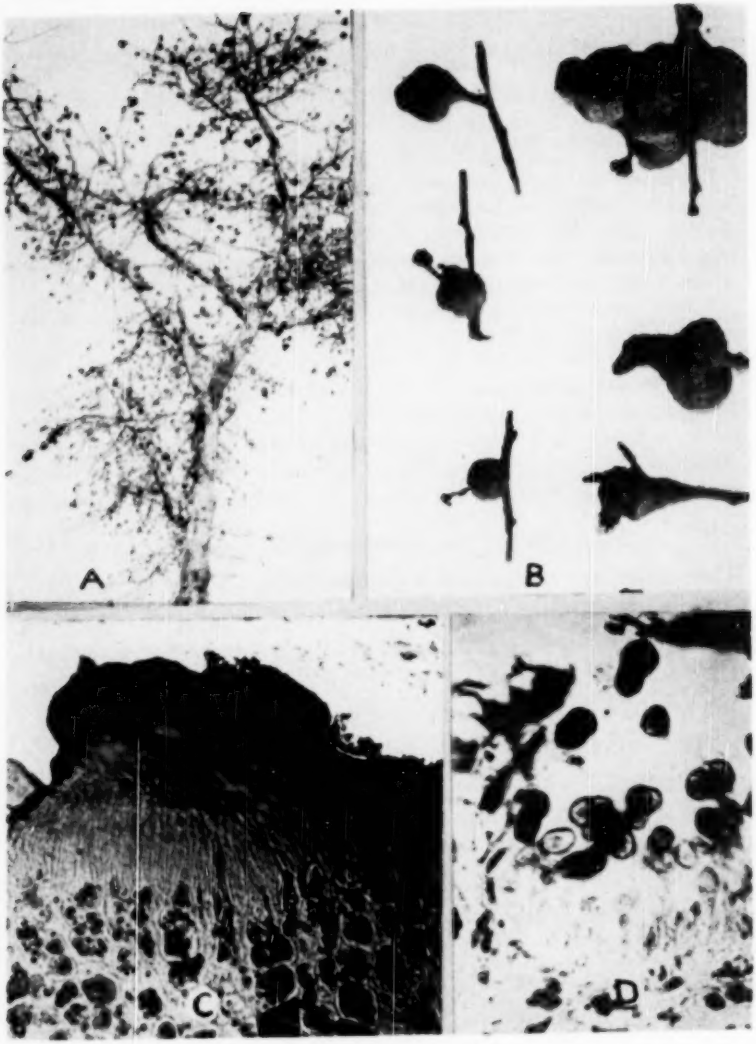


FIG. 8. *Hapalophragmiopsis ponderosum.*

was inclined to adduce much generic importance to such a mode of development. He did not observe the stages of teliospore germination since the spores had collapsed in the herbarium material.

The same rust species has been collected by the writer near Bangalore, Mysore, on *Bassia latifolia* and *B. Bourdilli* which is a new host species. The telia are formed in abundance in the months of December and January and the teliospores germinate immediately intra-sorum.

During the early stages of germination, the tip of the teliospore prolongates into a bud-like structure. There is no promycelium typical of other rusts having four-celled external promycelia. In contrast, the cylindric bud-like structure, into which the contents of the teliospore migrate, may become 1-septate and get separated off (FIG. 5). Further division takes place in this abbreviated promycelium, usually prior to their separation, and results in the formation of four cells. These are arranged in a linear row of four cells (FIG. 6) or, as in several instances, become disposed in a tetrahedral manner due to the plane of divisions (FIG. 7). No sterigmata have been observed and the promycelial cells round off into sporidia.

The tetrahedral disposition of the promycelial cells and their rounding off into sporidia is an interesting feature unknown so far among rust species that have been investigated. In *Coleosporium pulsatillae*, Weir (1912) found that during teliospore germination two intersecting vertical septations took place resulting in a basidium characteristic of the Tremellaceae.

Inoculation experiments have been carried out using the sporidia (scraped from mature sori), on young leaves of *Bassia latifolia* maintained in moist chambers for 48 hours. The inoculation experiments confirmed the autoecious nature of the rust, the pycnia developing on hypertrophied spots after 15 days. Mature pycnia are subcuticular, conoid, applanate, without conspicuous ostiolar paraphyses.

Further studies on such infection spots produced naturally showed the development of uredinoid aecia (primary uredia) accompanying the pycnia. These are subepidermal and without peridium or paraphyses. The aeciospores are identical with the

urediospores and borne similarly on clustered pedicels, thus conforming to the fasciculate type of development described by Kuhnholtz-Lordat (**1943**). The uredia (secondary uredia) that follow are hypophyllous and produced on small non-hypertrophied infection spots. The telia are associated with the uredia in early stages and later completely replace them.

The occurrence of uredinoid aecia in the life-cycle of the rust was overlooked by Mains who had access only to herbarium material. The aecia were described as being unknown for the genus. A reexamination of *Scopella gentilis* (Syd.) Mundkur & Thirumalachar and *S. aulicus* (Syd.) Mundkur & Thirumalachar (**1949**), occurring on species of *Mimusops* in India, has shown the occurrence of uredinoid aecia accompanying the pycnia.

UROMYCES LEPTODERMUS Syd. in Ann. Mycol. **4**: 430. 1906. Monogr. Ured. **2**: 334. 1910; Butler & Bisby, Sci. Monogr. No. 1, Counc. Agr. Res. India, p. 82, 1930.

Syn. *Uredo Isachnes* Syd. Ann. Mycol. **4**: 444. 1906.
U. Panici-prostrati Syd. Ann. Mycol. **4**: 444. 1906.
Nigredo leptoderma Arth. North Amer. Fl. **7**: 224. 1912.

Hab. On the leaves of *Brachiaria distachya* Stapf, Bangalore, 6–12–1946, leg. M. J. Thirumalachar; on *Cyrtococcum trigonum*, Yeshwantpur, Bangalore. 8–12–1945, leg. M. J. Thirumalachar.

UREDO CALLICARPAE Petch. in Ann. Roy. Bot. Gard. Peradeniya V, Part IV, p. 251, 1912; Monogr. Ured. IV, p. 420–421, 1924.

Hab. On the leaves of *Callicarpa lanata* Linn., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar.

Uredo Carissae sp. nov.

Uredia hypophylla, minuta, subepidermalia, primo sori evoluti supra epidermidem, et apparenter superficialis, paraphysata; paraphyses cylindricae, incurvatae formantes operculum nido similem 46–60 × 9–10 μ; urediosporae pedicellatae, oblongo-ellipsoideae, materiam aurantiaco-luteum continentes, germinationis poris indistinctis; episporium minute et dense verruculosum; sporae dimetientes 26–34 × 20–24 μ.

Habitat in foliis *Carissae* sp., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar.

Uredia hypophyllous, minute, subepidermal at first, sori formed above the epidermis and thus appearing superficial, paraphysate;

paraphyses cylindric, incurved, forming a nest-like covering 46–60 × 9–10 μ. Urediospores pedicellate, oblong-ellipsoid, with orange-yellow contents; epispore minutely and densely verruculose, with distinct germ pores. Spores measuring 26–34 × 20–24 μ.

On the leaves of *Carissa* sp., Kemmangundi, Mysore, 26–4–1949, leg. M. J. Thirumalachar. Type deposited in Arthur Herbarium, Purdue University, Lafayette, Indiana, U. S. A., Herb. C.M.I., Kew, England, and in Herb. Crypt. Ind. Orient, New Delhi, India.

AECIDIUM PARAMIGNYAE Racib. in Bull. l'Acad. des Sc. de Cracovie, Cl. des Sc. math. et nat. fevrier, p. 277, 1909.

Hab. On leaves of *Paramignya* sp., Balehonnur, Mysore, 4–3–1942, leg. M. J. Thirumalachar, 10–8–1944, leg. K. S. Gopalakrishna.

Pycnia and aecia are present in the material. The aeciospores measure 18–25 × 16–18.5 μ, and the peridial cells are thick-walled, 23–35 × 18–21 μ. *Aecidium Petchii* Sacc. & Trott. is reported from Ceylon on *Paramignya monophylla*, and the Sydows (**1924**) consider that it is probably identical with *A. Paramignyae* Racib.

In conclusion the writer wishes to express his gratitude to Dr. George B. Cummins, Arthur Herbarium, Purdue University, Lafayette, Indiana, for kindly going through the manuscript and for giving the benefit of valuable suggestions, and to Rev. Father Dr. H. Santapau, Professor of Botany, St. Xavier's College, Bombay, for kindly translating the diagnosis of the new genus and species into latin.

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DESCRIPTION OF FIGURES

FIGS. 1–4, *Hapalophragmium mysorense:* FIG. 1. Pycnium, × 400; FIG. 2. Uredinoid aecium, × 400; FIG. 3. Urediospores, × 950; FIG. 4. Teliospores, × 800.

FIGS. 5–7, *Scopella echinulata:* FIG. 5. Germination stages of the teliospore, × 1000; FIG. 6. Showing the linear arrangement of the promycelial cells, × 1000; FIG. 7. Tetrahedral disposition and rounding off of the promycelial cells, × 1000.

FIG. 8, A–D, *Hapalophragmiopsis ponderosum:* A, Infected tree of *Acacia leucophloea*; B, Developmental stages of the rust galls, × nat. size; C, Subepidermal pycnium, × 400; D, Section through telium, × 400.

THE NUTRITION OF MONOSPORIUM APIOSPERMUM

FREDERICK T. WOLF, ROBERT R. BRYDEN AND JOHN A. MACLAREN

(WITH 1 FIGURE)

INTRODUCTION

The disease of man known as maduromycosis, Madura foot, or mycetoma, is due to any one of a number of organisms included among both the actinomycetes and the filamentous fungi. Within the continental United States, the most frequent etiologic agent is *Monosporium apiospermum*.

Boyd and Crutchfield (**1921**) isolated from a case of maduromycosis an ascomycetous fungus which was described by Shear (**1922**) as *Allescheria Boydii*. In a Canadian strain of *M. apiospermum*, Emmons (**1944**) demonstrated the production of perithecia and ascospores similar to those of *A. Boydii*, thus proving that *Monosporium apiospermum* is the conidial stage of *Allescheria Boydii*. The name *M. apiospermum* is commonly retained, however, for those isolates in which the production of the *Allescheria* stage has not been observed.

Little information is available concerning the physiology of this pathogen. Boyd and Crutchfield (**1921**) noted that it could readily be grown in liquid media, and that a number of carbohydrates, including mannitol, galactose, xylose, rhamnose, mannose, lactose, sucrose, maltose and dextrose, were not fermentatively attacked. Benham and Georg (**1948**) have recently investigated the conditions necessary for the production of the perithecial stage in culture, concluding that media rich in organic nitrogen are favorable for formation of conidia but adversely affect perithecial production. These workers cultivated *M. apiospermum* upon a synthetic medium of dextrose agar containing 0.2 per cent asparagine as the only nitrogen source. Thus, the fungus is autotrophic insofar as its requirement for growth factors (vitamins, amino acids, purines and pyrimidines) is concerned.

In connection with observations on various fungi, it was noted that a culture of *Monosporium apiospermum* obtained from Dr. N. F. Conant, Department of Bacteriology, Duke University School of Medicine, grew nicely upon a Czapek's solution of the following composition: $NaNO_3$, 3.0 gm.; KH_2PO_4, 1.0 gm.; KCl, 0.5 gm.; $MgSO_4$, 0.5 gm.; $FeCl_3$, trace; and sucrose, 30 gm. per liter. It was therefore decided to examine certain phases of its nutrition in greater detail.

RESULTS

Carbon Sources: The utilization of a number of carbohydrates, alcohols, amino acids and other organic acids as sources of carbon for *Monosporium apiospermum* was studied using Czapek's solution prepared with the omission of sucrose. To this basal solution were

TABLE 1

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS SUCROSE) CONTAINING VARIOUS SUGARS, ALCOHOLS, AND ORGANIC ACIDS AS CARBON SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Control (no carbon)	0	Ethanol	++
Xylose	++++	Glycerol	++
Arabinose	++	Adonitol	+++
Rhamnose	0	Dulcitol	+++
Glucose	+++	Inositol	0
Levulose	++++	Sorbitol	0
Galactose	+++	Mannitol	0
Mannose	+++		
Sorbose	0		
Sucrose	+++	Acetic acid	++
Maltose	++	Malonic acid	+
Lactose	+	Succinic acid	++
Cellobiose	++	Fumaric acid	+
Melibiose	++	Malic acid	+
Trehalose	++++	Aconitic acid	+
Raffinose	++	Alpha keto Glutaric acid	+
Melezitose	++	Tartaric acid	++
Dextrin	+++	Citric acid	++
Soluble starch	+++		

added singly various carbon sources, each in an amount providing 400 mg. of carbon per 100 ml. of medium. Differences in the percentage of carbon among the various substrates were thus taken into account. Cultures were grown in 125 ml. Erlenmeyer flasks containing 50 ml. of solution, adjusted to an initial pH of 5.0.

Observations as to relative amounts of growth were made after two weeks at room temperature. The results are presented in tables 1 and 2.

It is apparent that *M. apiospermum* is able to utilize a wide variety of compounds as sources of carbon. In general certain of the sugars and amino acids are superior to the alcohols and dicarboxylic acids as carbon sources. Xylose, levulose and trehalose, among the sugars, and alanine, isoleucine and arginine among the amino acids proved to be the most favorable carbon sources. The organism is apparently unable to attack rhamnose, sorbose, inositol, sorbitol, or mannitol, nor can it utilize cystine, methionine, or histidine·HCl as a source of carbon.

TABLE 2

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS SUGAR) CONTAINING VARIOUS AMINO ACIDS AS CARBON SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Glycine	+	*l*-Arginine·HCl	++++
dl-Alanine	++++	*l*-Lysine·HCl	+
beta-Alanine	++	*l*-Aspartic acid	++
dl-Serine	+++	*l*-Asparagine	++
dl-Threonine	+	*l*-Glutamic acid	+++
dl-Valine	+++	*l*-Tyrosine	+++
l-Leucine	++	*dl*-Phenylalanine	++
dl-Isoleucine	++++	*l*-Tryptophane	+++
l-Cystine	0	*l*-Ornithine	++
dl-Methionine	0	*l*-Proline	+++
l-Cysteine·HCl	+	*l*-Hydroxyproline	+
l-Histidine·HCl	0		

Nitrogen Sources: Experiments to test the suitability of various amino acids as sources of nitrogen for *M. apiospermum* were conducted using a technique analogous to that employed in the experiments with carbon sources. In this case, however, Czapek's medium was modified by omission of $NaNO_3$, and the various amino acids were added in quantities providing 50 mg. of nitrogen per 100 ml. Controls were provided containing $NaNO_3$ or $(NH_4)_2SO_4$. The pH was adjusted to 5.0, and the results obtained after two weeks growth are presented in table 3.

It is apparent that each of the 23 amino acids tested is able to serve as a nitrogen source for *M. apiospermum*, glycine, valine,

glutamic acid and tryptophane being most satisfactory for growth of the fungus. Comparison of tables *2* and *3* indicates no correlation between the suitability of a given amino acid as a source of carbon and the suitability of the same compound as a source of nitrogen. *M. apiospermum* is able to utilize both organic (amino) nitrogen and inorganic nitrogen, the latter either as nitrate or ammonium.

Growth in Relation to pH: For studies of the influence of pH on growth of *Monosporium apiospermum*, Czapek's solution was prepared, lacking phosphate, which was supplied by means of McIlvaine's citric acid–Na_2HPO_4 buffers, covering the range of pH 2.2–8.0 at intervals of 0.4–0.6 pH unit. Each flask was prepared to contain 20 ml. of the proper buffer mixture (0.1 M citric acid and 0.2 M Na_2HPO_4) plus the ingredients (minus phosphate) for 50 ml. of Czapek's solution dissolved in 30 ml. The pH of each mixture was checked colorimetrically and the buffer concentration was found to be satisfactory. The cultures were observed after growth for two weeks at room temperature.

TABLE 3

GROWTH OF *Monosporium apiospermum* IN CZAPEK'S BASAL SOLUTION (MINUS $NaNO_3$) CONTAINING VARIOUS AMINO ACIDS, $NaNO_3$ OR $(NH_4)_2SO_4$ AS NITROGEN SOURCES

Substrate	Amount of growth	Substrate	Amount of growth
Glycine	++++	*l*-Lysine·HCl	++
dl-Alanine	+++	*l*-Aspartic acid	+++
beta-Alanine	+	*l*-Asparagine	+++
dl-Serine	++	*l*-Glutamic acid	++++
dl-Threonine	++	*l*-Tyrosine	++
dl-Valine	++++	*dl*-Phenylalanine	++
l-Leucine	+++	*l*-Tryptophane	++++
dl-Isoleucine	++	*l*-Ornithine	++
l-Cystine	+	*l*-Proline	++
dl-Methionine	+++	*l*-Hydroxyproline	+
l-Cysteine·HCl	+	$NaNO_3$	++++
l-Histidine·HCl	+++	$(NH_4)_2SO_4$	+++
l-Arginine·HCl	++		

No growth occurred at pH 3.0 or below. Growth occurred in all instances at pH 3.6 and above, increasing to a maximum at pH 7.0–7.6 and declining somewhat at pH 8.0.

To establish the alkaline limit for growth, additional experiments

were performed. Because the buffers customarily used in the pH range 8.0–12.0 contain ingredients such as borate or phthalate which might have effects on fungus growth other than those due primarily to pH, it was decided to employ unbuffered Czapek's solution adjusted to various pH values with concentrated NaOH,

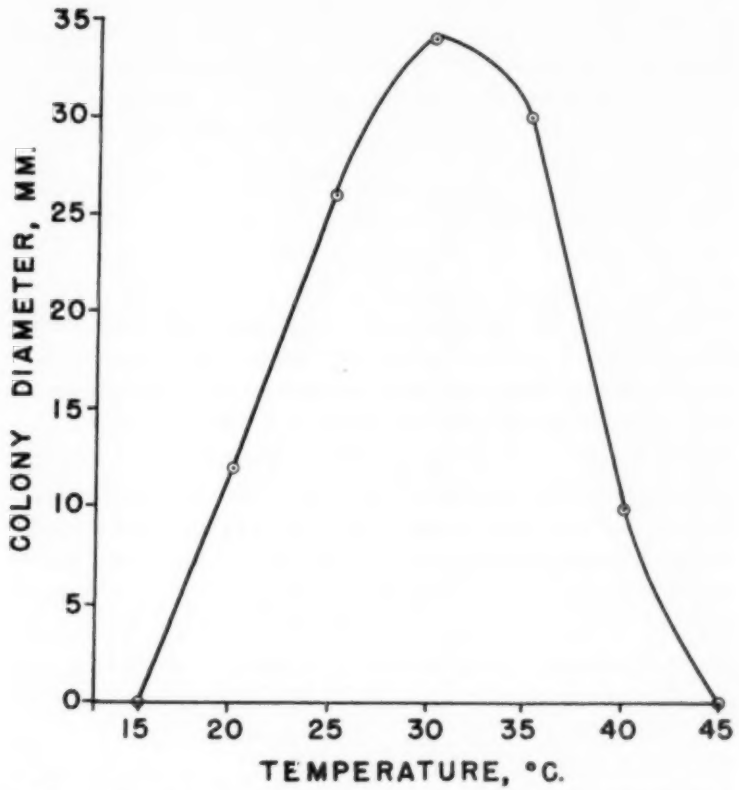

FIG. 1. *Monosporium apiospermum*. Growth in relation to temperature.

and checked by the glass electrode. These experiments indicated that *M. apiospermum* is able to grow up to pH 10.8, but not at pH 11.2 or beyond.

It is evident, therefore, that *M. apiospermum* can tolerate a very wide range of hydrogen ion concentrations, namely pH 3.6–10.8, with optimal growth on Czapek's medium at pH 7.0–7.6, which

approximates the pH encountered in its parasitic existence in tissues.

Growth in Relation to Temperature: Studies of the relation of temperature to growth of *M. apiospermum* were performed using plates of Czapek's solution solidified by the addition of 2% agar Plates were inoculated, and held at temperatures of 15°, 20°, 25°, 30°, 35°, 40° and 45° C. for one week, at which time the colony diameters were measured. The results are presented in figure 1.

No growth occurred at 15° or at 45° C. The minimal temperature for growth is therefore between 15 and 20° C. and the maximum between 40 and 45° C. The optimum is close to 30°, somewhat below body temperature.

DISCUSSION

The fact that *M. apiospermum* is autotrophic with respect to its requirements for growth factors is at variance with findings in a number of other fungi pathogenic to man, which have been shown to be deficient for one or more vitamins (Burkholder and Moyer, **1943**; Nickerson and Williams, **1947**). The present findings regarding the carbon nutrition of *Monosporium apiospermum* may be compared with those of Baker and Smith (**1942**) on *Coccidioides immitis*, a pathogenic fungus which has been carefully studied from this standpoint. Of 46 carbon compounds employed by these workers, excellent growth (++++) was obtained with glucose, fructose, mannose, galactose, maltose, cellobiose, trehalose, starch, dextrin, inulin, salicin, amygdalin, ethanol, glycerol, erythritol, mannitol, sorbitol, acetate, succinate, lactate and pyruvate. *C. immitis* does not utilize arabinose, sucrose, lactose, melezitose, inositol, tartrate or citrate, all of which with the exception of inositol are utilized by *M. apiospermum*. Interesting comparisons may also be made with *Aspergillus oryzae*, whose reactions toward 123 different carbon compounds has been studied by Tamiya (**1932**).

The unexpectedly good growth of *M. apiospermum* on the pentose sugar xylose is of particular interest. *M. apiospermum* has not been found in nature apart from clinical infections, known to be acquired from some exogenous source. The present finding indicates that

it may exist as a saprophyte in nature upon woody substrata or other plant material.

Little attention has been devoted to amino acids as sources of carbon for fungi. *Fusarium oxysporum* var. *lycopersici* and *Penicillium roquefortii* have recently been examined from this viewpoint by Gottlieb (**1946**), who obtained growth of both fungi with each of 21 amino acids tested, except for cysteine·HCl, cystine, methionine, and tyrosine. *M. apiospermum* is apparently unable to utilize cystine, methionine, or histidine·HCl as a carbon source but grows to a slight extent on cysteine·HCl, and well upon tyrosine. An important factor neglected in the present experiments was the possibility of effects upon growth due to the different isomers of an amino acid, since some of the amino acids used were the naturally occurring isomer and others were racemic mixtures.

An interesting feature of the experiments dealing with nitrogen sources was the finding that *M. apiospermum* is able to utilize each of the 23 amino acids tested as a nitrogen source, while several of these compounds, as noted above, were unavailable to the organism as sources of carbon. The ability to utilize inorganic as well as organic nitrogen would place *M. apiospermum* in Group II of the classification of Robbins (**1937**). Among the pathogenic fungi, the normal form of *Trichophyton mentagrophytes* requires organic nitrogen, although pleomorphic strains can utilize ammonium but not nitrate nitrogen (Robbins and Ma, **1945**). According to Baker and Smith (**1942**), *Coccidioides immitis* is, like *M. apiospermum*, non-exacting in this respect, being able to utilize nitrate, ammonium or organic nitrogen.

The finding that *M. apiospermum* is able to grow throughout a wide range of pH is reminiscent of the results of Leise and James (**1945, 1946**) with *Epidermophyton floccosum* and several species of *Trichophyton*. These organisms were able to grow at pH 10.5, under conditions which suppressed common contaminants.

The finding of an optimum pH for growth of *M. apiospermum* at pH 7.0–7.6 is an apparent exception to the statement that fungi prefer acid substrata. Yet a similar result was obtained by Cross (**1948**) with the yeast-like phase of *Histoplasma capsulatum*, which showed an optimum on several media in the range pH 7.2–7.6, a finding which may prove typical of other systemic fungi.

SUMMARY

Monosporium apiospermum has been grown in Czapek's synthetic nutrient solution. The organism is able to utilize a wide variety of sugars, alcohols, amino acids and organic acids as sources of carbon. Good carbon sources include xylose, glucose, levulose, galactose, mannose, sucrose, trehalose, dextrin, starch, dulcitol, adonitol, alanine, serine, valine, isoleucine, arginine·HCl, glutamic acid, tyrosine, tryptophane and proline. Rhamnose, sorbose, inositol, sorbitol, mannitol, cystine, methionine and histidine·HCl are not available to the organism as sources of carbon.

M. apiospermum is non-exacting in its nitrogen requirement, being able to utilize nitrate, ammonium or amino nitrogen. Growth occurred upon each of 23 amino acids tested as a nitrogen source. Glycine, alanine, valine, leucine, methionine, histidine·HCl, aspartic acid, asparagine, glutamic acid and tryptophane were better nitrogen sources than the other amino acids tested.

This fungus can grow within the pH range 3.6–10.8, with an optimum pH of 7.0–7.6.

Growth of *M. apiospermum* occurs between a lower temperature limit at 15–20° C. and an upper limit at 40–45° C., with an optimum at approximately 30° C.

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A STUDY OF AN ISOLATE OF BREVILEGNIA FROM NEW CALEDONIA

T. W. JOHNSON, JR.[1]

(WITH 1 FIGURE)

During the progress of a study of fungi isolated from soil samples sent by Professor J. T. Buchholz from New Caledonia to the Mycological Laboratories, Department of Botany, University of Illinois, a species of the genus *Brevilegnia* was obtained which appeared to be different from any of the hitherto described species in the genus. An investigation by Salvin (**4**) of an isolate of *Brevilegnia* obtained from Cuba led him to conclude that specific and varietal characteristics in the genus were so variable that only two species appeared valid, and that these should be transferred as a sub-genus to the genus *Thraustotheca*. It seemed obvious, therefore, that a designation of the New Caledonia isolate as a new species had to be done with a consideration of the possibility of variation.

The conclusions presented by Salvin were arrived at by the study of one isolate only, and the observations were not supported by a comparative study of isolates whose characteristics under standardized cultural conditions were those of previously described species. Since such comparisons were lacking, it is possible that only his isolate could be characterized by such extreme variability, and that more stable forms existed which should be recognized as valid species. Therefore, a study was initiated (a) to determine if the New Caledonia isolate possessed variability comparable to Salvin's isolate, and (b) on the basis of results obtained, to determine if it could be regarded as representing a variant of one of the accepted species of *Brevilegnia* or as a new species.

[1] The author wishes to express his sincerest gratitude to Professor Leland Shanor, for his guidance and helpful suggestions in the course of the study, and in the preparation of this paper. The author's present address is, Department of Botany, University of Michigan, Ann Arbor, Michigan.

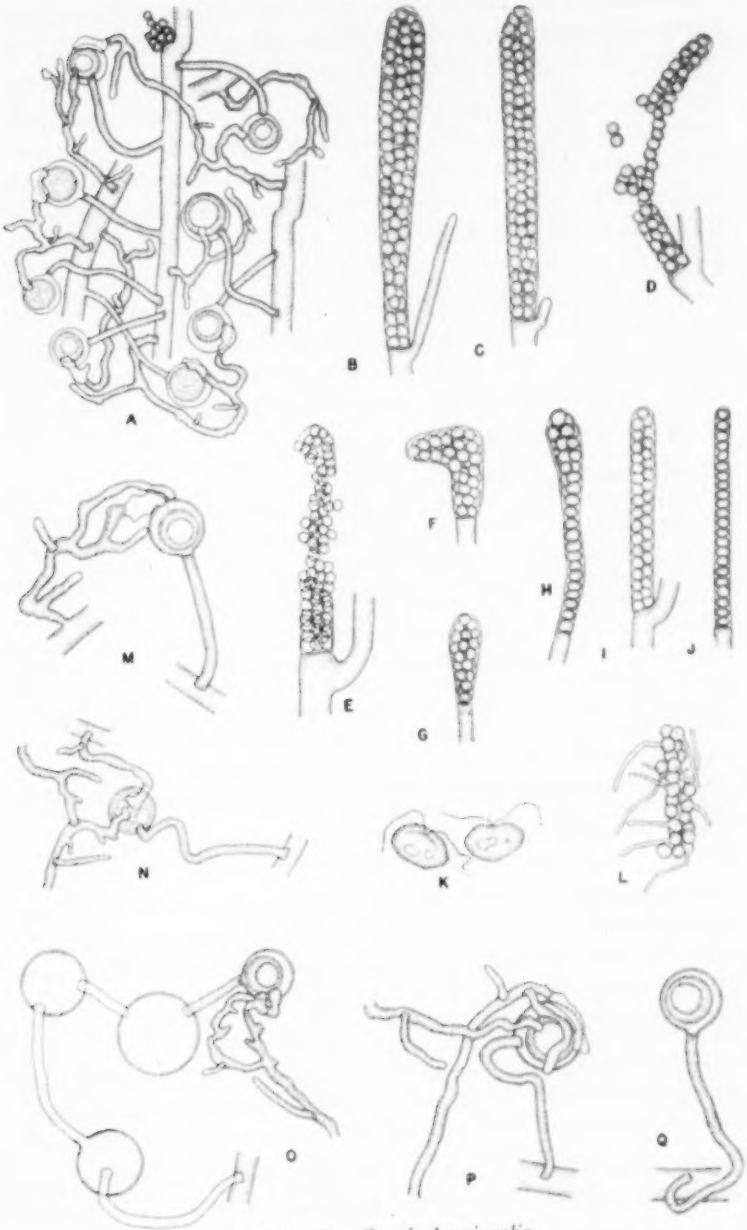

FIG. 1. *Brevilegnia longicaulis.*

Each isolate employed in this study was derived from a single, germinated zoospore, and was maintained in culture growing on boiled, split hempseed in 30 cc. of sterile, charcoal-filtered, distilled water at a temperature of 22 ± .3° C. Assignment of specific names to all isolates of other species of *Brevilegnia* at hand was done on the basis of the characteristics of those isolates when grown in such cultures, since the original descriptions of species assigned to the genus were based either wholly or in a large part upon isolates grown in this manner. The details of culture methods employed for the study of possible variations under differing environmental conditions will be described under the section dealing with experimental studies. This paper presents the results of these studies, and in view of these results, the New Caledonia isolate is believed to represent an undescribed species.

Brevilegnia longicaulis sp. nov.[2] Mycelium spissum, breve, quod abundantes in *Cannabis sativae* seminibus gignitus. Hyphis plerumque non ultra 5.0 mm. longis, crassis, et ad basim ramosis; mediis hyphis plerumque 24.7 μ diam. Sporangiis copiosis, clavatis in longis cylindricalibus, 167–301 μ longis, 22.1–26.8 μ diam.; terminalibus, cymosis. Sporis plus v. minus angularibus, plerumque 9.1 μ diam., quae aut in situ germinant aut sporas motiles secundarias proferunt. Oogoniis copiosis, singulis et ad ramuli partem extremans affixis; 24.8–29.0 μ diam.; sphaericis; tunicas leves, tenues, hyalinas, et nulla papilla alterave parre prominente deformatas habentibus. Oosporis 22.5–26.0 μ diam., sphaericis, quae singulae singulis in oogoniis ita gignuntur ut oogonia non impleant et paulo ab oogonii centro absint. Episporio levi, crasso. Antheridiis diclinis, longis, haud multum ramosis, quae plerumque singula, nonnumquam bina, terna, vel quaterna in oogoniis gignuntur sed interdum omnino desunt. Nullis gemmis.

Hab.: ad terram humosam, Köe Dumbra, New Caledonia, September 23, 1947.

Mycelium depauperate, forming a dense growth on hempseed. Hyphae usually not more than 5.0 mm. long, averaging 24.7 μ in diameter near the center; delicate and branching. Sporangia abundant, terminal, clavate to long-cylindrical, 93–375 μ long by 21.8–53.6 μ in diameter, averaging 167–301 × 22.1–26.8 μ; generally 3–5 spores wide, not branching, not producing spores in a single row either in part or in the whole; secondary sporangia arising by cymose branching. Encysted spores spherical to angular, 6.2–11.0 μ in diameter, averaging 9.1 μ; encysting within the

[2] Latin diagnosis prepared with the assistance of Professor R. P. Oliver, Department of Classics, University of Illinois.

sporangium, and escaping upon disintegration of the sporangial wall, then leaving the cysts as motile spores; only rarely escaping from the cysts while these are still united in a partly disintegrated sporangium, and then leaving a partial false-net cluster of cysts; occasionally germinating *in situ*. Oogonia abundant, spherical, 22.5–33.3 μ in diameter, generally 24.8–29.0 μ; arising singly on long, slender, lateral stalks; wall smooth, thin, hyaline, unpitted; immature oogonia frequently proliferate until an antheridium becomes attached. Oospores 1 to each oogonium, spherical, and not filling the oogonium; eccentric, with a large oil drop entirely surrounded by protoplasm; 20.1–27.5 μ in diameter, generally 22.5–26.0 μ. Antheridial branches diclinous, long, irregular, and branched; never observed androgynous, and seldom not developing; one, rarely two to four antheridia to the oogonium; antheridial cell broadly attached to the oogonium; antheridial tubes not observed. Gemmae lacking.

From soil collected from the edge of a brook in a ravine, approximately 5 miles east of Köe Dumbra, New Caledonia, September 23, 1947, by Professor J. T. Buchholz.

Slides of preserved material from the type culture are being deposited in the herbaria of the University of Illinois, and the University of Michigan.

For purposes of distinguishing *Brevilegnia longicaulis* from other members of the genus which have been described, we have used living cultures, collected in Illinois by Dr. E. S. Beneke, of *Brevilegnia unisperma*, *B. unisperma* var. *delica*, *B. unisperma* var. *montana*, *B. unisperma* var. *littoralis*, and *B. diclina*, and have compared our isolate with the original descriptions of *B. bispora*, *B. linearis*, *B. subclavata*, and *B. megasperma* (**1, 2, 3**). In view of the possible variations which may exist in sporangial characters, prime consideration was given to fundamental differences in sexual reproductive structures. Salvin has indicated that even in his variable isolate, he considered such features as the type of antheridium and general morphology of the oogonium to be genotypically determined. These structures, then, it would seem, are more constant than sporangial characteristics for determining specific differences. With this consideration, and on the basis of the comparisons made, *Brevilegnia longicaulis* differs from any of the previously described species and varieties in the genus.

EXPERIMENTAL STUDIES

Six control cultures, each consisting of one-half of an infected hempseed, were employed for each phase of the experimental study. These cultures were propagated as single spore isolates in 30 cc. of sterile, charcoal-filtered, distilled water at a temperature of $22 \pm .3°$ C. Comparisons of the experimental colonies and the control colonies were made when both were of approximately the same age. Although only one culture on hempseed was used for each environmental condition, all experiments were repeated several times. Unless otherwise specified, the results herein reported are based on the average of 200 observations or measurements in each culture observed. Growth under any of the conditions not discussed below was similar to growth in the controls.

Effect of environment on sporangial morphology: As suggested by the procedure outlined by Salvin in his investigation of *Brevilegnia* isolate C–2, *Brevilegnia longicaulis* was grown in varying amounts of sterile, charcoal-filtered, distilled water, with and without aeration, at temperatures ranging from 10 to 35° C. (at intervals of 5° C.), and in water containing the staling products from several cultures of the isolate. The volumes of water used were 1, 5, 10, 25, 50, 100, 300, 500, 1000, 1500, and 2000 cc. The staling water employed was obtained by filtering, through a porcelain filter, water in which cultures of *B. longicaulis* had previously been grown. Twenty colonies, each one week old, were placed in 50 cc. of sterile, charcoal-filtered, distilled water, and allowed to develop for a period of 2 weeks. This procedure was repeated several times until sufficient staling water had been obtained. Cultures were grown in 25, 50, 100, and 200 cc. of this water, without aeration, at a temperature of 25° C. The effects of temperatures of 2, 5, and 7° C. on sporangia of cultures grown in volumes of water of 25, 50, 100, 200, 300, 500, and 1000 cc. were also studied. In all cases, observations were made on sporangial shape and size.

In the case of cultures grown in 1 and 5 cc. of water, without aeration, at temperatures of 25, 30, and 35° C., sporangia were somewhat shorter than those of the control cultures. The range of the measurements of these sporangia was 67.5–181.5 × 22.5–23.1 μ, as compared with the range of 171.5–297.0 × 22.1–27.6 μ for the

controls, and they were typically clavate in shape. In quantities of water from 10 to 500 cc., without aeration, at temperatures of 25, 30, and 35° C., sporangia showed some variation, the most noticeable being a slightly smaller diameter, with a tendency, in 115 of the sporangia measured, to be mostly 2 spores wide (FIG. *I*). Sporangia in these cultures measured 187.5–202.0 × 19.1–21.5 μ, and were usually long-cylindrical in shape. In volumes of water above 500 cc., without aeration, sporangia were similar to those in the controls, except for a few sporangia in the cultures grown at 25° C. in 1000 cc. of water, these being abnormal in that the tips were bent as indicated in figure *F*. As far as could be observed, temperature in the range employed did not appear to have any pronounced effect on sporangial morphology.

In the experiments involving aeration in addition to the volumes of water and temperatures as listed above, cultures grown in 25, 50, and 100 cc. of water at a temperature of 25° C. produced sporangia which, in 2.2 per cent of the 300 sporangia observed, were longer and more cylindrical than those in non-aerated cultures. This degree of variation appears negligible, however, when compared with the variation of 60.8 per cent which Salvin obtained with isolate *C–2*, when grown under comparable conditions. In the temperature range between 10 and 25° C., in aerated water, regardless of amount, sporangia were shorter, but remained essentially clavate in shape. The significance of this variation at lower temperatures in aerated cultures has not been determined. The variation is interesting, however, in view of the lack of such variation when the water was not aerated.

Cultures of *Brevilegnia longicaulis* were also grown in 4000 cc. of water, with aeration, at a temperature of 25° C. Examination of 255 sporangia from these cultures indicated that the only variation induced in these structures was a decrease in their diameter. The average diameter of these sporangia was 17.3 μ, but in respect to length and shape, the sporangia were similar to those in the controls. Repetitions of the tests employing 4000 cc. of aerated water revealed the following variations: (a) the production of sporangia which were 1-spore wide in the basal half, and 2–3 spores wide in the apical half, in about 16 per cent of 205 sporangia observed (FIG. *H*), and (b) the production of sporangia which were 1-spore

wide throughout their entire length (FIG. *J*). This extreme narrowing, not observed in any other cultures, occurred in approximately 12 per cent of 200 sporangia observed.

The study on *Brevilegnia C–2* showed that in that isolate, at least, temperatures below 10° C. inhibited the full development of the sporangia, and any visible development was limited to a simple swelling of the hyphal tips. Cultures of *B. longicaulis* held at 2° C. failed to develop any sporangia, and after two weeks in this environment, examination revealed that no swelling of the hyphal tips had occurred. Attempts to obtain growth from these cultures by hyphal tip transfers onto MP-5 Agar were unsuccessful, and it is presumed that the mycelium was killed by the low temperature. At 5 and 7° C., however, the hyphal tips showed some swelling in the form of elongate enlargements suggesting the shape of the typical clavate sporangia. Development beyond this stage was not observed.

Staling water was utilized in an attempt to induce variations in sporangial shape. Salvin reported that sporangia of *Brevilegnia C–2*, developed on cultures growing in concentrated staling water, without aeration, for 10–14 days, were almost "exclusively short and clavate," with only a "few having the spores arranged in a linear series" (**4**, p. 42). The number of spores in the sporangia in his cultures ranged from 4–16. Of the 160 sporangia of *B. longicaulis* observed in cultures grown in 50 cc. of unaerated staling water at a temperature of 25° C., 74.2 per cent were typical in size, whereas the remainder were shortened, ranging from 31.0–87.5 μ in length (FIG. *G*). The number of spores in these sporangia was large in comparison with the number of spores reported for *Brevilegnia C–2*.

Effect of the environment on the production of gemmae: Cultures of *Brevilegnia longicaulis* were grown for 12 days in 25 cc. of staling water (obtained by the same procedure outlined for the study of sporangial variations) at a temperature of 25° C., but in no cultures were gemmae produced. Similarly, older cultures of *B. longicaulis*, growing in the water in which they were originally placed, did not form gemmae. Salvin, however, was consistently able to induce the formation of gemmae by the use of concentrated staling water, even though they were not formed, in his isolate,

on cultures in "pure water" (**4**, p. 44). The results obtained with our isolate would tend to indicate that, in this species at least, the formation of gemmae may be genetically controlled and not influenced by an environmental factor.

Effect of the environment on antheridial formation: In the report on the investigation of *Brevilegnia C–2*, the amount of available oxygen was found to be the factor contributing most to variations in antheridia. Again utilizing Salvin's procedure, colonies of *B. longicaulis* were suspended at various depths of water from the surface to 12 inches, at intervals of 1 inch, and temperature was maintained at 25 ± .5° C. Standard glass cylinders were used which measured 35 mm. inside diameter. After 10 days the cultures were examined for variations in the antheridia, both in percentage formed and for origin. No attempt was made to determine, quantitatively, the actual degree of aerobiosis in the culture tubes.

Of the oogonia of *B. longicaulis* formed at the 10-inch level, 61.3 per cent had antheridia attached, 38.7 per cent had no antheridia. Of the oogonia produced in the cultures growing at the 5-inch level, 63.9 per cent had antheridia attached, the remainder lacked them, and of the oogonia produced at surface level, 69.6 per cent had antheridia. For comparison, control cultures produced oogonia 72.0 per cent of which had antheridia, whereas the remaining 28 per cent of the oogonia lacked these structures. Briefly, the results of Salvin showed that with increasing depth at which the mycelium of *Brevilegnia C–2* developed, fewer antheridia were formed. A culture of *Brevilegnia C–2* growing at the surface of a 10-inch column of water had 82 per cent of the oogonia with antheridia attached, but a culture growing at the 10-inch depth of the column had only about 4 per cent of the oogonia with antheridia.

The effect of the degree of aerobiosis on the origin of the antheridial branches was determined by observations of these same cultures. The percentages of antheridia developing diclinously, androgynously, or not at all, were very similar in the 10-inch, 5-inch, and surface levels to the percentages developing in the standardized control cultures: diclinous, 71.1 per cent; androgynous, 0.0 per cent, and not developing, 28.9 per cent. These results agree with those obtained by Salvin in that he reported that characters such as type of antheridium (diclinous or androgynous)

were not proven to vary with the environment, but rather, were apparently determined genetically.

Although not included in Salvin's study of *Brevilegnia C–2*, the characteristics of *B. longicaulis* when grown in more natural environments were investigated. Fifteen separate single spore isolates of *B. longicaulis* were grown in 15 samples of water and debris. These samples were selected from such diverse locations as cold, clear, running water; cold, stagnant water, and warm stagnant water. After two weeks in these "natural habitats," the cultures were observed, particularly for changes in the distinguishing characteristics. With the exception of minor variations in the morphology of sporangia (these were usually longer, and a greater number of them had the encysted spores germinating *in situ*), the features of the fungus were not obviously altered.

DISCUSSION

In the recent paper by Salvin (**4**), reporting the effects of various environmental factors on the characteristic morphological structures that distinguish the genus *Brevilegnia*, as represented by his isolate, *Brevilegnia C–2*, the variation in sporangial size and shape, formation of gemmae, and the presence or absence of antheridia, were evaluated. Particular attention was paid to the duplication of the various sporangial characteristics that have distinguished most of the previously described species belonging to the genus, and Salvin concluded from his results that only two of all the species and varieties that had been reported should be retained as specific entities. *Brevilegnia bispora* was retained, but all of the others were regarded as merely representing variations of *B. unisperma* that could be induced by varying the environment in which the cultures were grown. Furthermore, Salvin proposed the reduction of *Brevilegnia* to a sub-genus of *Thraustotheca*, designating the species in the sub-genus as *Thraustotheca bispora* and *T. unisperma*.

On the basis of the results of a study of an isolate of *Brevilegnia*, herein reported as *Brevilegnia longicaulis*, we are not prone to accept all of the conclusions reached by Salvin. In the first place, our isolate, *B. longicaulis*, does not exhibit the wide variability in sporangial and other characters which Salvin reported for *Brevi-*

legnia C–2. Within reasonable limits, our isolate seems to be a a very stable organism. Should the same be true for the other species of *Brevilegnia*, there would be no justifiable reason for grouping these under the specific name *Brevilegnia unisperma*. Before Salvin's conclusion in this regard is accepted, it would be highly desirable to study the other species of the genus to determine if they follow the pattern of variability exhibited by his one isolate. Indeed, *Brevilegnia C–2* may only exhibit a degree of variability not infrequently encountered in selected isolates representing other species of fungi.

Secondly, we see nothing gained by reducing *Brevilegnia* to subgeneric rank under these circumstances. When the genus was established by Coker and Couch (1), the similarity of certain of the sporangial characters in *Brevilegnia* and *Thraustotheca* was recognized. Other characteristics, however, such as the single oospore, the depauperate nature of the mycelial development, and the behavior of the spores in *Brevilegnia*, were regarded as the basic characters separating this genus from the genus *Thraustotheca*. Salvin's results have in no way altered this original hypothesis. Therefore, we prefer to retain *Brevilegnia* as a genus.

On the basis of our study of *Brevilegnia longicaulis*, we are able to concur fully with Salvin that the type of antheridium, the general morphology of the oogonium, size of oospores, and the method of sporangial proliferation are stable features, and it is primarily on the basis of these that the isolate we have studied has been distinguished from the other species of the genus.

SUMMARY

A new species of *Brevilegnia* is described as *Brevilegnia longicaulis*. The fungus was recovered from a soil sample obtained along a ravine near Köe Dumbra, New Caledonia. It is characterized by clavate to long-cylindrical sporangia renewed by cymose branching, motile zoospores, diclinous antheridia, proliferating oogonia, and oospores which are consistently larger than those in species and varieties of *Brevilegnia* previously described, with the exception of *Brevilegnia megasperma*.

The fungus was subjected to extensive experimentation in an attempt to induce specific variations in the morphology of the spo-

rangia, the origin of the antheridia, and to induce the formation of gemmae. Variations obtained were slight, and not sufficiently pronounced to conclude that this species was merely a variant of any other species.

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DESCRIPTION OF FIGURES

Brevilegnia longicaulis. FIG. *A.* Portion of mycelium growing on hempseed, showing long oogonial stalks, and diclinous, irregular and branched antheridia. × 840. *B.* Mature sporangium of the characteristic clavate type. × 840. *C.* Mature sporangium of the typical long-cylindrical type. × 840. *D.* Mature sporangium showing disintegration of wall, and liberation of encysted spores. × 840. *E.* Sporangium in which some of the encysted spores have been liberated normally, the remainder having emerged from their cysts, leaving a partial false-net sporangium. × 840. *F.* A bent sporangium formed in a culture grown at 25° C. in 1000 cc. of unaerated water. × 840. *G.* A short-clavate sporangium formed in a culture grown at 25° C. in 50 cc. of unaerated staling water. × 840. *H.* A modified sporangium with spores in a single row in the basal half, observed in cultures grown at 25° C. in 4000 cc. of aerated water. × 840. *I.* A narrow-cylindrical sporangium 2 spores wide, formed in a culture grown at 30° C. in 100 cc. of unaerated water. × 840. *J.* A modified sporangium 1 spore wide, formed in a culture grown at 25° C. in 4000 cc. of aerated water. × 840. *K.* Motile stage of zoospores after emergence from cyst. × 1980. *L.* A cluster of encysted zoospores, germinating *in situ.* × 840. *M.* Typical mature oogonium showing diclinous antheridial branches, attachment of antheridia, and mature oospore. × 1180. *N.* Immature oogonium with attached diclinous antheridial branch. × 840. *O.* Proliferation of an oogonium, showing the ultimate, mature one with a diclinous antheridium. × 1180. *P.* Mature oogonium with 2 attached antheridial branches. × 1180. *Q.* Mature oogonium which has developed parthenogenetically. × 1180. All figures made with aid of a camera lucida.

THE ACTIVITY IN VITRO OF CYCLOHEXIMIDE (ACTI-DIONE[1]) AGAINST FUNGI PATHOGENIC TO PLANTS

ALMA J. WHIFFEN

(WITH 1 FIGURE)

The demonstration by Dr. Irma Felber and Dr. Charles Hanner (**1948**) that powdery mildew (*Erysiphe polygoni* DC.) on bean plants in the greenhouse could be controlled by the antibiotic cycloheximide in concentrations from five to ten parts per million has aroused interest in the effectiveness of cycloheximide against other plant pathogens. Earlier, cycloheximide had been shown to be active against a number of yeasts but only slightly active against the filamentous fungi that cause disease in animals (Whiffen, **1948**). In this paper the spectrum of antibiotic activity of cycloheximide *in vitro* is extended to cover twenty-nine species of fungi pathogenic to plants.

MATERIALS AND METHODS

With the exception of cultures obtained from culture collections, as noted, all species tested were freshly isolated from diseased plants. Isolation of the pathogenic fungi was greatly aided by the addition of streptomycin, in concentrations from 100 to 300 micrograms per milliliter, to plates of Blakeslee's medium (Difco malt extract 20 grams, Bacto-peptone 1 gram, dextrose 20 grams, agar 20 grams, distilled water 1000 milliliters). These concentrations of streptomycin suppressed or inhibited the multiplication of the majority of contaminating bacteria without having any inhibitory effect upon the growth of the fungi.

Eleven species were obtained for us by Dr. Ralph Lewis from individuals and culture collections as follows: *Sclerotinia fructicola*,

[1] Cycloheximide has been assigned as the generic name for the compound β-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide for which The Upjohn Company adopted "Acti-dione" as its trademark.

Alternaria solani, and *Alternaria oleracea*, S.E.A. McCallan, Boyce Thompson Institute; *Sclerotium rolfsii*, W. D. McClellan, Bureau of Plant Industry, Beltsville; *Pythium debaryanum* and *Diplodia zeae*, Benjamin Koehler, University of Illinois; *Macrosporium sarcinaeforme*, J. G. Horsfall, Connecticut Agricultural Experiment Station; *Physalospora tucumanensis* Light Strain and Dark Strain, C. W. Edgerton, Louisiana State University; *Venturia inaequalis* 365–4, G. W. Keitt, University of Wisconsin; *Diaporthe citri*, L. J. Klotz, Citrus Experiment Station of the University of California, Riverside, California.

Crystalline cycloheximide was diluted in plates of D.P.Y. agar medium to give concentrations of 100.0, 40.0, 20.0, 10.0, 5.0, 2.5, 1.25 micrograms per milliliter and 1.0, 0.5, 0.25, 0.125, 0.062 microgram per milliliter. The composition of D.P.Y. medium is as follows: dextrose 10 grams, Bacto-peptone 5 grams, Difco yeast extract 1 gram, agar 20 grams, distilled water 1000 milliliters; pH 6.8. Spore suspensions of the fungal pathogens were prepared from ten-day-old agar slant cultures on Blakeslee's medium and streaked on the surface of plates containing cycloheximide. Those fungi that did not sporulate on agar, *e.g.*, *Pythium debaryanum*, *Rhizoctonia* sp., and *Sclerotium rolfsii*, were grown on agar plates and a disc of mycelium was cut from the margin of the colony for inoculum. The concentration at which there was complete inhibition of growth was recorded after 48 hours of incubation at 24° C. and again after 72 hours. The tests were replicated four times with four different lots of spores.

To determine the effect upon the rate of mycelial growth, cycloheximide was diluted in D.P.Y. agar to cover a range of concentration from 0.0075 to 4.0 micrograms per milliliter, using a dosage ratio of 2.0. Duplicate plates were inoculated in the center with an agar disc, four millimeters in diameter, cut from the edge of a forty-eight-hour-old culture of *Pythium debaryanum*. The plates were incubated at 30° C. and at intervals of 12 to 24 hours, the margin of the colony was marked along previously ruled radii. The rate of growth in terms of millimeters per hour was determined for each concentration of cycloheximide and for the control lacking

cycloheximide and from these data the percentage of retardation of mycelial growth from that of the control was calculated. The experiment was replicated five times. This method of determining toxic effect on the growth of mycelium was suggested by Bateman (**1933**).

EXPERIMENTAL RESULTS

In table 1 are shown the concentrations of cycloheximide at which the growth of thirty-three plant pathogens was completely inhibited

TABLE 1

THE *In Vitro* EFFECT OF CYCLOHEXIMIDE UPON THIRTY-THREE PHYTOPATHOGENIC FUNGI

Organisms	Disease	Micrograms per milliliter completely inhibiting growth	
		48 hours	72 hours
Class Phycomycetes			
Order Peronosporales			
Pythium debaryanum	Damping Off	10.0	20.0
Class Ascomycetes			
Order Pezizales			
Sclerotinia fructicola No. 19	Brown Rot of Peach	2.5	5.0
Sclerotinia fructicola B	Brown Rot of Peach	10.0	20.0
Sclerotinia fructicola BTI	Brown Rot of Peach	10.0	20.0
Diplocarpon rosae	Black Spot of Rose	5.0	10.0
Order Myriangiales			
Elsinoe veneta	Anthracnose of *Rubus*	20.0	40.0
Order Hypocreales			
Gibberella saubinetii	Barley Scab	10.0	20.0
Order Sphaeriales			
Physalospora tucumanensis L.S.	Red Rot of Sugar Cane	5.0	10.0
Physalospora tucumanensis D.S.	Red Rot of Sugar Cane	5.0	10.0
Diaporthe citri	Canker	2.5	2.5
Endothia parasitica	Chestnut Blight	5.0	20.0
Gnomonia veneta	Anthracnose of Sycamore	10.0	20.0
Gnomonia leptostyla	Anthracnose of Hickory	10.0	20.0
Guignardia aesculi	Leaf Blotch of Horse Chestnut	5.0	10.0
Venturia inaequalis 365–4	Apple Scab	5.0	10.0
Class Basidiomycetes			
Order Ustilaginales			
Ustilago tritici	Loose Smut of Wheat	0.062	0.125
Ustilago zeae	Common Smut of Corn	2.5	10.0
Fungi Imperfecti			
Cercospora apii	Leaf Spot of Celery	20.0	20.0
Heterosporium iridis	Leaf Blight of Iris	2.5	5.0
Cladosporium fulvum	Leaf Mold of Tomato	0.125	0.25
Cladosporium paeoniae	Leaf Mold of Peony	5.0	10.0
Macrosporium sarcinaeforme	Leaf Spot of Clover	10.0	20.0
Alternaria solani	Early Blight of Potato	20.0	40.0
Alternaria solani BTI	Early Blight of Potato	10.0	10.0
Alternaria oleracea BTI		10.0	20.0
Ramularia pastinaceae	Leaf Spot of Parsnip	40.0	100.0
Diplodia zeae	Diplodia Disease of Corn	1.25	1.25
Colletotrichum lagenarium	Anthracnose of Cucurbits	2.5	10.0
Colletotrichum phomoides	Anthracnose of Tomato	10.0	10.0
Fusarium lycopersici	Tomato Wilt	40.0	100.0
Sphaeropsis elysii	Leaf Spot of Black Pine	0.062	0.125
Sclerotium rolfsii	Stem Rot	1.25	2.5
Rhizoctonia sp.	Dollar Spot	1.25	1.25

after 48 and 72 hours of incubation. With the exception of the three non-sporulating fungi mentioned above, the inhibition observed was predominantly that of spore germination. It will be noted that in almost every case a concentration of cycloheximide that prevented germination of spores after 48 hours was insufficient to do so after 72 hours. In an experiment carried to 92 hours there was growth at still higher concentrations than at 72 hours. At any

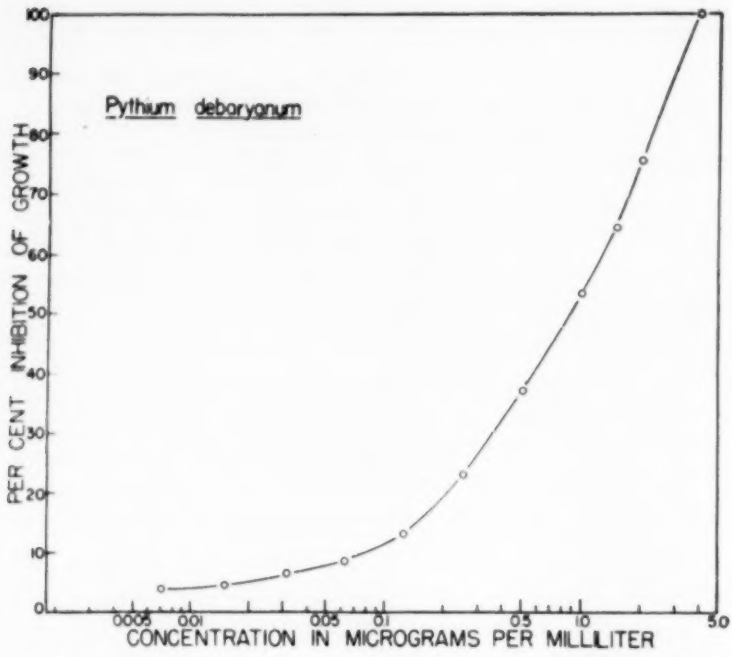

FIG. 1. Dosage-response curve of *Pythium debaryanum* to cycloheximide.

given time partial inhibition of growth could be observed over a range of two to sixteen fold differences in dosage. The growth of all species and strains of fungi tested was completely inhibited after forty-eight hours of incubation by a concentration of cycloheximide of 40 micrograms per milliliter or less. Of the thirty-three organisms, two were inhibited by 40, three by 20, ten by 10, seven by 5, five by 2.5, three by 1.25, and three by less than 1 microgram of cycloheximide per milliliter.

The dosage-response curve of *Pythium debaryanum* to cycloheximide is shown by figure 1 in which the dosage has been plotted on a logarithmic scale against the percentage of mycelial inhibition. When plotted in this manner, the toxicity curve is not a straight line; nor is a straight line obtained when probit response is plotted against logarithm of dosage. The dosage response curve of *P. debaryanum* on logarithmic probability paper has a double slope, curvature concave upwards, and resembles the toxicity curve found by McCallan *et al.* (**1941**) for Bordeaux Mixture against *Glomerella cingulata*.

DISCUSSION AND CONCLUSIONS

Cycloheximide has an inhibitory effect against a wide variety of phytopathogens including one order of Phycomycetes, four orders of Ascomycetes, one order of Basidiomycetes and twelve genera of the Fungi Imperfecti. In view of its widespread activity against fungi pathogenic to plants, it is surprising that cycloheximide apparently is ineffective against the filamentous fungi that parasitize animals.

Including cycloheximide, approximately twenty-two antibiotic substances have been described in the literature as possessing antifungal properties. Of these, only one other than cycloheximide has been extensively tested against phytopathogenic fungi. An impure preparation of antimycin, an antibiotic produced by *Streptomyces* sp., inhibited the growth of eight different species of fungi pathogenic to plants in concentrations of less than two micrograms per milliliter and five other species in concentrations from three to two hundred and fifty micrograms per ml. (Leben and Keitt, **1948**). Pure antimycin may be expected to be more highly active *in vitro* than cycloheximide.

The *in vitro* activity of cycloheximide against fungi pathogenic to plants described in this paper is merely an indication of its possible usefulness in the control of plant disease. In addition to determining the toxicity of cycloheximide to pathogenic fungi under field conditions, it will also be necessary to know the toxicity of cycloheximide to the affected plants. Experiments directed to this end have been begun (Vaughn *et al.*, **1949**).

SUMMARY

The antibiotic cycloheximide inhibited the growth *in vitro* of thirty-three species and strains of phytopathogenic fungi within a range of concentration varying from 0.125 to 100.0 micrograms per milliliter. The effect of cycloheximide upon the mycelial growth of *Pythium debaryanum* was determined and the dosage response curve plotted.

ACKNOWLEDGMENT

The assistance and interest of Dr. Ralph Lewis in the collection and identification of the pathogenic fungi is gratefully acknowledged.

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THE GENUS MARSSONINA ON QUERCUS AND CASTANEA

PAUL L. LENTZ *

(WITH 1 FIGURE)

A specimen of a fungus causing oak leaf spots was recently sent for determination to the Bureau of Plant Industry by Professor A. A. Dunlap of the Department of Plant Pathology, A. & M. College of Texas. The spots seemed to be caused by a species of *Marssonina*, but the organism was not *M. martini* (Sacc. & Ell.) Magn., the common species on oak. Eventually it was learned that some specimens similar to this fungus had previously been placed in the mycological collections of this Bureau as *Gloeosporium septorioides* Sacc., and that others had mistakenly been determined as *M. martini*. Examination of additional specimens and a search through the mycological literature revealed much confusion concerning this organism. Since this fungus is apparently of rather frequent appearance on oak, the following discussion is presented with the hope that some of the confusion may be eliminated and that attention may be directed to the fact that there are two species of *Marssonina* common on oak in the United States.

In 1884, Winter described *Marsonia quercina* from a specimen collected in Missouri by Demetrio. The fungus sent from Texas by Professor Dunlap belongs to the same species as that described by Winter. The original description by Winter is clear and adequate, except for the necessity of extending the size range of the conidia and other slight modifications.

The fungus described as *Marsonia quercina* causes spots on oak leaves similar to those caused by *M. martini*. The margin may be somewhat more definite and of a slightly darker reddish-brown color than the margin of the spot caused by *M. martini*, but this is not always true. The most reliable macroscopic character that

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may be used for distinguishing the present fungus from *M. martini* is the host upon which it is found. Of 45 specimens of *M. martini* examined during this study, all were on leaves of species belonging to the white oak group. (*Cf. Marsonia quercus* Pk. below.) The 38 specimens of Winter's *Marsonia quercina* studied all occurred on leaves of species belonging to the black oak group.

Marssonia ochroleuca (Berk. & Curt. ex Pk.) Humph. ex Seym. (*cf.* below) on *Castanea* resembles the "black" oak fungus even more closely than does *M. martini*, and it is surprising that specimens from oak do not seem to have been given the name of the chestnut fungus. Both *Marssonia ochroleuca* and the "black" oak fungus differ from *M. martini* by having longer, more slender conidia which are not constricted at the septum. The acervuli of the chestnut fungus seem to be somewhat more shallow than those of the "black" oak fungus, but this is not constant. Although the chestnut fungus and the "black" oak fungus are very much alike in every respect, with only vague differences easily accounted for by the difference in host, the two are nevertheless here treated as separate species. This is in keeping with the recognition of the usual association of leaf spot fungi with narrowly restricted hosts and also with tradition, as indicated by the failure of other workers to identify the "black" oak fungus as *Marssonia ochroleuca*.

Part of the confusion concerning the identity of the present fungus no doubt results from the variable nature of the acervulus, an unfortunate phenomenon so far as taxonomy is concerned. It may vary from a sub-sphere to a saucer in form. At maturity it is usually opened broadly and lacks any conspicuously defined wall of the type which would permit it to be considered a pycnidium. Usually the fungus elements at the base of the conidiophores form a thin basal stratum of more or less isodiametric, hyaline and inconspicuous cells, although occasionally there may be an increase in density and definiteness and a slight darkening in some areas, particularly at the base of the fructification. While the acervulus of *M. martini* is confined to the region just beneath the epidermal layer, the fructification of the "black" oak fungus may be confined to this area or may form a deep cavity in the leaf tissue, hypophyllous fructifications frequently forming a cavity extending to the lower margin of the palisade layer of leaf tissue. At maturity the

fructification is usually broadly open, and the conidia are freed by splitting of the leaf epidermis over the acervulus.

An additional source of confusion concerning this fungus has been the failure of some workers to see the single septum of the conidium, a structure which frequently is difficult to see by the usual staining methods. By the following method, used during this study, it may be observed with ease: A bit of the acervulus was obtained by use of a needle filed down to a chisel-like edge at the tip. This particle was placed on a slide, moistened with alcohol, swelled with 2 per cent KOH solution, stained with phloxine, and crushed beneath a cover slip by gentle tapping on the latter. Measurements of conidia were then made and were found to differ very little if at all from measurements of conidia well moistened in water. The septum could usually be discerned with some difficulty when the conidia were being measured. Following this examination, the cover slip was removed and a drop of glycerine acidulated with acetic acid was placed on the bit of crushed acervulus. The cover slip was replaced, and examination of the conidium then revealed that the cytoplasm had pulled away slightly from the septum, and that the latter could be seen distinctly.

During this study, it was found that specimens of the "black" oak leaf fungus had been placed in the mycological collections of this station under the following designations: *Marsonia* quercina* Wint., *Marssonia quercina* Wint. var. *major* Overh., *Gloeosporium septorioides* Sacc., *Marssonina quercus* (Pk.) Magn., *Marssonina martini* (Sacc. & Ell.) Magn., *Septoria quercicola* Sacc., *Septoria quercus* Thuem., and *Cylindrosporium microspilum* Sacc. & Wint. Of these, the first three represent synonyms of the new combination presented below for the "black" oak fungus. The others are misdeterminations.

To make the matter clear, the following list is presented of species which examination of specimens and/or consultation of literature has shown to be entirely distinct from the fungus occurring on leaves of trees in the black oak group: *Marssonina martini* (Sacc. & Ell.) Magn., *Marssonina quercus* (Pk.) Magn., *Marsonia matteiana* Sacc., *Phleospora hanseni* Bub., *Ascochyta quercus* Sacc. &

* *Marsonia* and *Marssonia* are earlier, now invalid, names for the present genus *Marssonina*.

Speg., *Ascochyta quercuum* (Cke.) Sacc., *Cylindrosporium microspilum* Sacc. & Wint., *Cylindrosporium quercus* Sorok., *Cylindrosporium quercinum* J. Carter, *Septoria quercicola* Sacc., *Septoria quercina* Desm., *Septoria quercus* Fckl., *Septoria quercus* Thuem., *Septoria ocellata* (Lev.?) Sacc., and *Septoria neglecta* Earle.

A portion of the type collection of *Marsonia quercus* Pk. was studied through the courtesy of Dr. H. D. House of the New York State Museum. In a letter accompanying the specimen, Dr. House expressed doubt that the host was really *Quercus ilicifolia* Wang., as stated in Peck's (**1885**) original description of the species. He suggested that the type material was actually collected from two hosts: *Q. rehderi* Trel. and *Q. prinoides* Willd. Examination of a portion of the type collection on leaves of what appear to be two different species of oak shows *Marsonia quercus* Pk. to be identical with *M. martini* (Sacc. & Ell.) Magn. Thus *Marssonina quercus* (Pk.) Magn. is a synonym of *M. martini* (Sacc. & Ell.) Magn. Previous experience, noted above, of finding *M. martini* only on leaves of species of the white oak group leads to the conclusion that both of the species of oak in the Peck collection must belong to this group as, judging from the configuration of the leaves, they well may.

Magnus (**1906**) has shown that *Marssonia* Fisch. is not valid as the name of a genus of fungi; therefore he changed the name to *Marssonina* Magn. During the study of the fungus known as *Marssonia ochroleuca*, on *Castanea*, it was noted that a transfer to *Marssonina* had apparently never been made. Heald (**1926**, p. 29) has published a photograph of this fungus, beneath which is the caption "Leaf spot (*Marssonina ochroleuca*) of chestnut." There is no further discussion, and this hardly constitutes publication of a new combination. It is probable that Heald merely substituted the new generic term for the old without concerning himself over the fact that the combination had really never been made. *Septoria ochroleuca* was published as a Berk. & Curt. species by Peck (**1873**). Berkeley (**1874**) later published the same species, but his description is antedated by Peck's. On specimens 173a and 173b of Seymour and Earle's Economic Fungi is the designation "*Marsonia ochroleuca* (B. & C.) Humphrey in litt." In Seymour's Host Index of the North American Fungi (**1929**), the species is

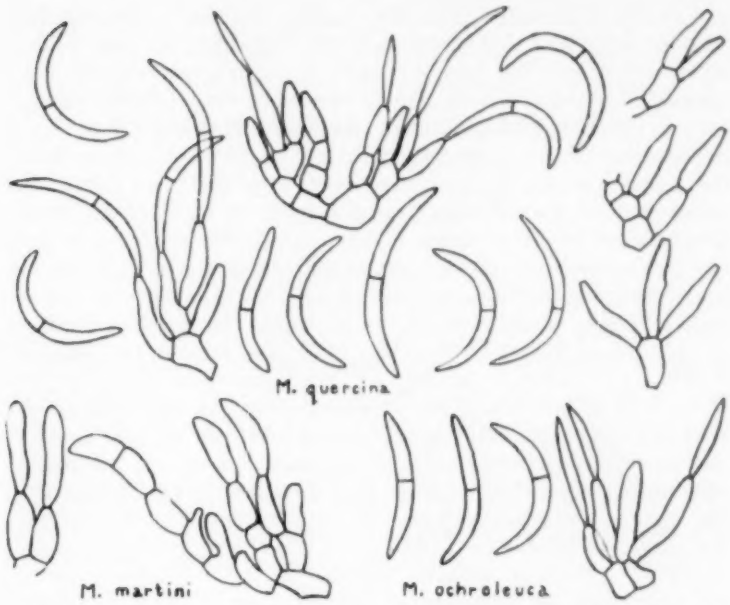

FIG. 1. Conidiophores and conidia of *Marssonina quercina* (top), *M. martini* (bottom, left), and *M. ochroleuca* (bottom, right). Magnification, ×1000.

included, among other synonyms, as "*Marssonia ochroleuca* (B. & C.) J. E. Humphrey." For this fungus, the following new combination is proposed: **Marssonina ochroleuca** (Berk & Curt. ex Peck) comb. nov.

In addition, the following new combination, with synonymy and description, is presented for the fungus which occurs on species of the black oak group:

Marssonina quercina (Wint.) comb. nov.

Marsonia quercina Wint. Hedw. **23**: 171. 1884; also in Rab.-Wint. Fungi Eur. no. 3085. 1884.

Gloeosporium septorioides Sacc. R. Instituto Veneto Atti. Ser. 6. **3**: 726. 1885.

Gloeosporium septorioides Sacc. var. *major* Ell. & Ev. Jour. Myc. **1**: 111. 1885.

Marssonia quercina Wint. var. *major* Overh. Mycologia **18**: 32. 1926.

Leaf spots amphigenous, few and scattered to numerous and sometimes confluent, orbicular to angular-orbicular, (0.5)1–4.5

(5.5) mm. in diameter, the upper surface dull tan [1] (intermediate between light buff and warm buff of Ridgway), or more rarely faded clay color, the margin usually extremely narrow but conspicuous, chestnut brown, verona brown to warm sepia, or seal brown, with the leaf sometimes discolored up to 2–3 mm. from the margin, the lower surface dull tan to cinnamon buff or faded clay color, with the margin often less conspicuous than that of the upper surface, concolorous with the spot, or more often Prouts brown, snuff brown to bister, or seal brown; the acervulus usually one but occasionally several to the spot, usually hypophyllous but occasionally epiphyllous, subglobose with a broad opening to concave or nearly plane, covered by the leaf epidermis, then opening by splitting of the latter, 50–150 μ from base to apex and 150–250 (300) μ in diameter, lacking a conspicuous pycnidium-like exterior wall, sometimes slightly darkened toward the base, but usually with the conidiophores arising from a thin base of hyaline more or less isodiametric cells; the conidiophores of two to several hyaline cells, unbranched, or more often branched one to three times; the apical, conidium-bearing cells of the conidiophores hyaline, slenderly obclavate, (6.5)10–13 × (1.5)2–3 μ, bearing a single conidium at the truncate apex; the conidia hyaline, smooth, acerose to falcate, non- or 1-septate as seen attached to the conidiophore, 1-septate when discharged, not constricted at the septum, (14.5)16–26(30) × 1.5–2(2.5) μ, most often 18–23 × 1.5–2 μ, oozing out of the acervulus in an amber brown mucilage which often forms a thin resinous-appearing coating over the leaf spot when dry.

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[1] With the exception of the terms "reddish-brown," "tan" and "amber brown," the terms used in this paper to describe colors are those used in Ridgway (1912).

PERMANENT STAINED MYCOLOGICAL PREPARATIONS OBTAINED BY SLIDE CULTURE

ROLAND W. RIDDELL *

(WITH 17 FIGURES)

The cultivation of fungi on microscopic slides, either by van Tieghem cell or by culture chamber methods, is a routine laboratory procedure. All such procedures commonly employed have certain inherent disadvantages and limitations. These include the amount of time and labor involved in preparation, and the difficulty due to the presence of culture medium when staining and making permanent preparations. These objections are largely obviated by a method to be described below which has, in addition, the asset of providing adequate aeration during culture. The method is superior to all that have been tested, and is quite similar to one used at the London School of Hygiene and Tropical Medicine. It is presented herewith since it is believed that it should be widely employed in mycological laboratories.

About 10 ml. of any chosen agar medium is melted and poured into a sterile 9 cm. Petri dish to form a layer 2 mm. deep. After the medium solidifies, preferably by refrigerator cooling, 1 cm. squares are ruled out over the whole plate using a sterile dissecting needle and a flamed glass rod (FIG. 1). This is done rapidly to reduce chances of contamination while the lid is displaced. Using sterile forceps, a flamed microscopic slide is placed under cover of a Petri dish lid, the inside of which was also flamed. A flamed cover slip is placed upon this slide to overlap at one end. An agar square is now lifted out of the Petri dish with precautions to prevent contaminations (FIG. 2), and is transferred rapidly to the center of the cooled slide (FIG. 3). A needlepoint inoculum of spores, or a small bit of mycelium, is placed at the center of each

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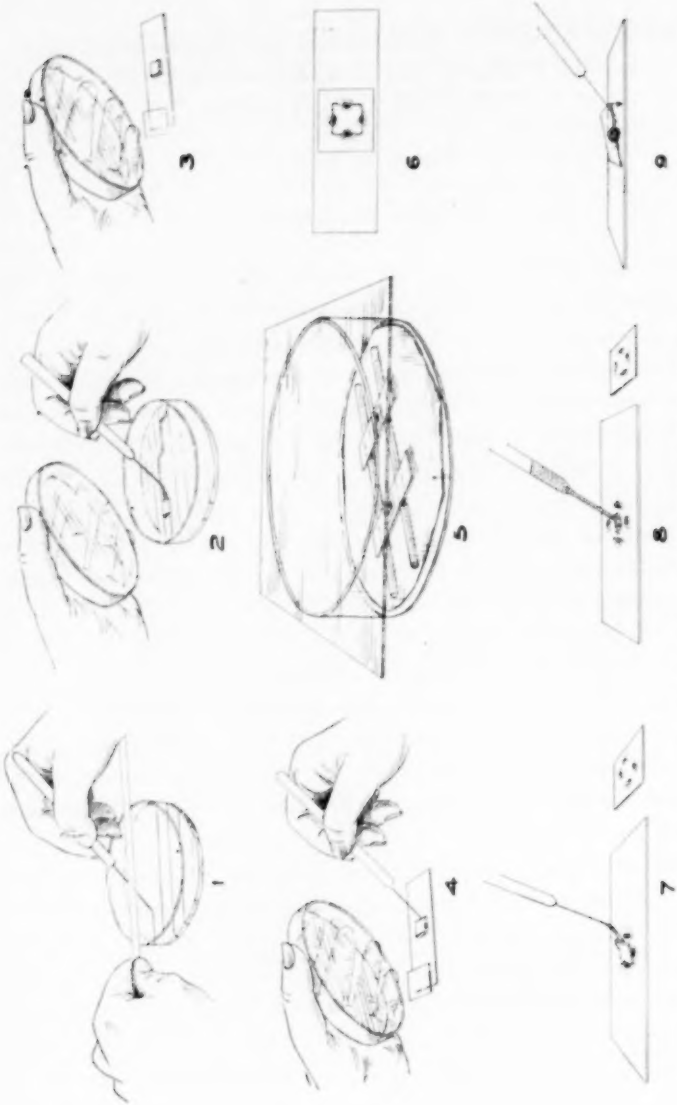


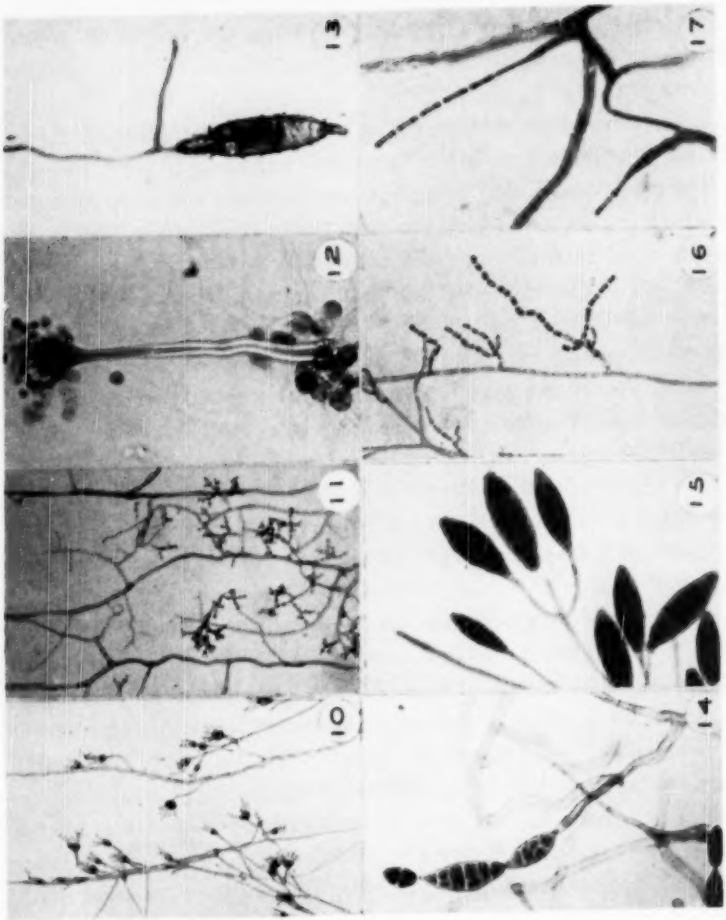
FIGS. 1–9. Technique for making slide cultures.

edge of the agar block over the whole 2 mm. depth at this point (FIG. 4). With sterile forceps the cover slip is placed centrally upon the upper surface of the agar square, and the slide is then transferred to a moist chamber (FIG. 5). Care is taken that the cover slip is not moved during growth of the fungus as when changing from the 16 mm. to the 4 mm. objective in microscopic inspections.

The earliest suitable time before excessive sporulation occurs is chosen to terminate growth. It is rarely necessary to allow growth to extend beyond 3 mm. from the cover slip edge, and often growths of about 1 mm. wide give the best results (FIG. 6). The cover slip is lifted vertically from the agar block without twisting movement and is placed aside, culture uppermost. Beginning at one corner the lifter is gently inserted under the agar block so as to lift it completely off the slide (FIG. 7). Thereby the agar and most of the fungus growth are discarded and all that remain on both slide and cover slip are four areas of growth joined by scanty lines of mycelium. A drop of 95 per cent ethyl alcohol is applied to the center of each of the rings of growth and is allowed to flow outwards to wet the fungus (FIG. 8). Just before it has completely evaporated a drop of lactophenol blue [1] is applied as was the alcohol. A clean cover slip is gently lowered upon the slide culture to spread the dye outwards over the damp mycelium (FIG. 9). Similarly a clean slide is applied to the cover slip preparation and is then quickly inverted. The slides are left undisturbed overnight after which any dye expressed is absorbed by strips of blotting paper applied to the cover slip edges. Fixation of the cover slips can now be obtained by application of colored nail lacquer to the corners. When this has dried, more lacquer is used to seal off the cover slip edges and, when dry, it may be trimmed with a razor blade. Two permanent stained preparations are thus made from each slide culture.

The agar concentration (*e.g.*, about 1.5 per cent Bacto-agar in Sabouraud's medium) should be suitable to allow a square to be lifted out cleanly without fraction. A lifter can be made by ham-

[1] Lactophenol blue: phenol crystals 20 gm., lactic acid 20 cc., glycerol 40 cc., distilled water 20 cc. Dissolve by gently heating and add 0.05 gm. cotton blue (methyl blue).



FIGS. 10-17. Photomicrographs of various fungi from slide cultures.

mering out the red-hot end of a dissecting needle bent to an obtuse angle. The Petri dish of unused agar blocks may be stored in the refrigerator. If the agar block is too thick little growth becomes attached to the glass, whereas if too thin it shrinks during the period of fungus growth and becomes difficult to remove. The moist chamber is constructed from a glass dish in which are laid two layers of paper towelling soaked in 20 per cent glycerol in water, and the slide cultures are supported above the paper by glass rods. The rim of the dish is sealed with vaseline and a glass plate is laid upon it. The chamber need not be sterile as is demonstrated by the rarity of contaminants in the cultures. The glycerol is incorporated to prevent "fogging" of the slides. It may be necessary to add more glycerol solution to the paper during culture but excessive wetting is avoided. There should be no marked shrinkage of agar during incubation. The application of alcohol to the fungus prevents inclusion of air bubbles and facilitates staining, but an excess present when the dye is added gives irregular results. The size of the lactophenol blue drop is adjusted so as just to fill a cover slip area. It is desirable that a centrifugal movement of alcohol and dye over the area occupied by the fungus is achieved, and as little movement of the cover slip as possible. Where sporulation is excessive the cover slip preparation is the better of the two, as it is relatively free from deposited spores. A *Penicillium* species is a suitable organism with which to practice the technique.

The method provides a simple means of observing aerial and submerged mycelial growth of fungus and developing fruiting structures in situ. Permanent stained preparations are made which demonstrate general fungus distribution (FIGS. 10 and 11), stages of spore germination (FIGS. 12 and 13), and the development of fruiting structures (FIGS. 14 and 15). It is at the moment being used to investigate the effects of chemicals and antibiotics present in the medium upon cultural morphology (FIG. 16). Conidial apparatus may be studied in detail (FIG. 17). It provides a rapid routine diagnostic aid to supplement observation made by teasing apart preparations on a slide. In medical mycology, it is possible to use for demonstration slides small portions of infected hair or skin scales as inocula.

DESCRIPTION OF FIGURES

Photomicrographs of various fungi from slide cultures stained with lactophenol blue: (10) *Penicillium* sp. (11) *Neurospora sitophila*. (12) *Aspergillus* sp.—germination and sporulation. (13) *Microsporum canis*—macroconidium germination. (14) *Alternaria* sp.—conidium formation. (15) *Microsporum gypseum*—stages in macroconidium development. (16) *Polyporus versicolor*—Oidium production on monokaryotic mycelium. (17) *Endoconidiophora* sp.—conidiophores.

A NEW GENUS OF THE CHOANEPHORACEAE

LELAND SHANOR, ADRIAN W. POITRAS, AND R. K. BENJAMIN

(WITH 12 FIGURES)

The family Choanephoraceae is one of the smaller families of the order Mucorales but one of considerable interest because certain of its species are parasitic on higher plants and, within the family in the various genera, the spores may be formed in Mucor-like sporangia, in sporangioles, or as monosporous sporangioles which are usually referred to as conidia. The genera are separated primarily on a combination of the different sporulating structures produced and from two to as many as six genera are included in the family by different authors (Fitzpatrick, **1930**; Zycha, **1935**; Naumov, **1939**). The six genera included by Zycha (**1935**) are: *Blakeslea* Thaxter, *Choanephora* Currey, *Cunninghamella* Matruchot, *Rhopalomyces* Corda, *Thamnocephalis* Blakeslee, and *Sigmoideomyces* Thaxter. In *Blakeslea* many-spored sporangia and sporangioles are produced but no conidia; in *Choanephora* many-spored sporangia and conidia are formed but no sporangioles; in *Cunninghamella, Rhopalomyces, Thamnocephalis,* and *Sigmoideomyces* only conidia are known. Naumov (**1939**) includes only *Choanephora* and *Blakeslea* in the Choanephoraceae and segregates *Cunninghamella, Sigmoideomyces* and *Thamnocephalis* in a separate family, the Cunninghamellaceae. Zygospores in *Blakeslea* and *Choanephora,* where known, are formed between the tips of twining branches and the species are heterothallic. Zygospores in *Cunninghamella,* where known, are formed more as are those of *Mucor* rather than between tips of twining branches. Zygospores in *Rhopalomyces,* in *Thamnocephalis,* and in *Sigmoideomyces* are unknown, a fact which has influenced some students to exclude these genera from the Mucorales.

Early in March 1949 there appeared on a collection of rabbit dung taken in Brownfield Woods near Urbana, Illinois, a very in-

teresting mucoraceous fungus which formed zygospores of the Mucor type in great abundance and produced asexual spores in sporangioles which arise on the end of long stalks from inflated tips of erect hyphae characteristic of the Choanephoraceae. This fungus was isolated in single spore culture and has been grown since on a number of culture media. Sporangiole formation on a capitate vesicle on erect sporangiophores has been the only type of asexual reproduction obtained. In the way in which it reproduces asexually, this fungus appears most closely related to *Blakeslea* and *Choanephora* but cannot be included in either genus as defined at present, and it differs strikingly from either in the way in which zygospores are formed. The type species, which at present represents the only one known in this new genus, is homothallic. The genus is being named in honor of Professor William Chambers Coker.

Cokeromyces Shanor, gen. nov.*

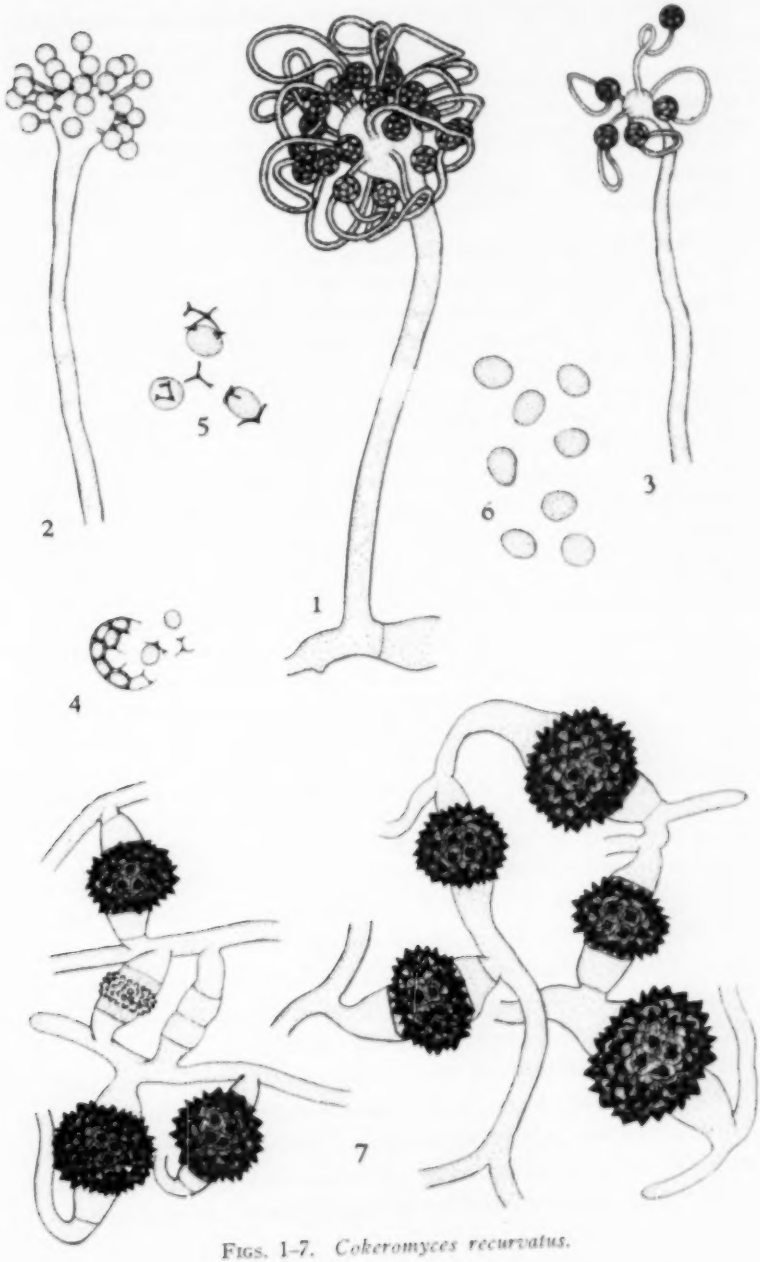
Sporangiolis globosis natis e stipitibus quae affatim nascuntur in summis sporangiophorum tumoribus terminalibus et capitatis; sporis nigris quae minime sunt striatae nec appendiculas habent radiatus; sporangiis aut conidiis nullis. Zygosporae inter ramos conexivos nascuntur eodem modo ac zygosporae Mucoris vel Cunninghamellae, et non inter tortilium ramorum apices ut Blakesleae vel Choanephorae zygosporae.

Sporangioles produced abundantly on stalks over the surface of terminal capitate swellings of sporangiophores; spores dark and not possessing radiating delicate cilium-like appendages; sporangia and conidia absent. Zygospores produced between short, relatively straight copulating branches as in *Mucor* or *Cunninghamella* and not between tips of twining branches as in *Blakeslea* or *Choanephora*.

Cokeromyces recurvatus Poitras, sp. nov. (FIGS. 1–12)

Sporangiolis globosis quae nascuntur in cacuminibus longorum stipitum qui e vesiculis terminalibus crescunt sporangiophorum ramis plerumque carentium quae se usque ad 500 μ e substramine porrigunt; sporangiophoris plerisque 9 μ in diametro; vesiculis terminalibus 12.6–31.5 μ (vulgo 21 μ) in diametro; sporangiolorum stipitibus 60–120 μ (vulgo 80 μ) longis, 2.2 μ fere in diametro, recurvatis atque tortilibus cum maturuerunt; sporangiolis globosis 8.4–12.6 μ (vulgo 10 μ) in diametro, parietes foris leves sed intus reticulatos habentibus,

* We are indebted to Professor Revilo Oliver, Classics Department, for the preparation of Latin diagnoses.



FIGS. 1–7. *Cokeromyces recurvatus.*

5–30 vel plura e vesicula terminali nascuntur; sporis non unius magnitudinis ovoidibus vel incompositis, leves parietes habentibus, 2.5–4.5 μ, quorum duodecim vel plura usque ad viginti sporangiolum fert. Sporangia et conidia plane desunt. Zygosporae quae affatim nascuntur sive inter ramos conexivos e hyphis superficialibus crescentes sive ex zygosporarum iam maturatarum suspensoribus, hispidos habent parietes e quibus prominentia retusa vel teretia vel acuta usque ad 4 μ se porrigunt. Hae zygosporae globosae fuscae et rubidae sunt cum maturuerunt, 33.5–54.5 μ (vulgo 43–45 μ) in diametro; homothallicae.

Mycelium rather slow-growing on culture media, producing reproductive structures very quickly; sporangioles formed at tips of long recurved stalks which arise from a terminal vesicle of usually unbranched sporangiophores which extend up to about 500 μ above substrate, usually about 325 μ high; average diameter of sporangiophores about 9 μ; terminal vesicles 12.6–31.5 μ in diameter, usually about 21 μ; sporangiole stalks recurved and twisting when mature, variable in length, 60–120 μ long, usually about 80 μ, about 2.2 μ in diameter with wall thicker and darker than that of the vesicle; sporangioles essentially globose, 8.4–12.6 μ in diameter, usually about 10 μ, outer wall smooth, inner wall surface often reticulate because of a deposit, 5–30 or more formed from each vesicle, usually about 20–22; spores not uniform in size, ovoid to somewhat irregular, commonly measuring about 2.5 by 4.5 μ, not striate, smooth-walled, except outer ones in sporangioles which may have a reticulate deposit on outer surface, usually about 12–20 per sporangiole. Conidia and many-spored sporangia absent. Zygospores produced in great abundance between copulating branches which arise usually from superficial hyphae, sometimes from outgrowths of sporangiophores, and very often from suspensors of more mature zygospores, walls rough because of prominent, variable, blunt, rounded or sharply pointed projections which are up to 4 μ in length; zygospores globose, brown when mature, measuring 33.5–54.5 μ in diameter including projections, usually about 43–45 μ; homothallic.

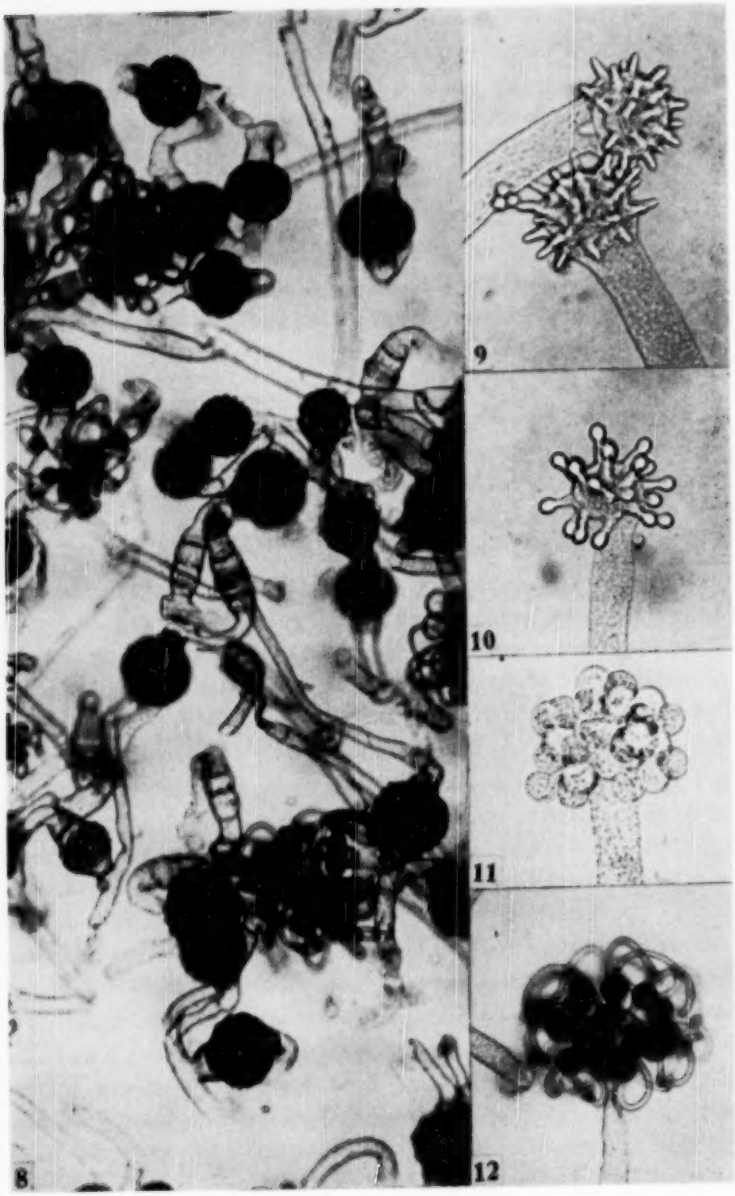
Developed on rabbit dung collected in Brownfield Woods, Urbana, Illinois, March 17, 1949. Type material deposited in the Mycological Collections of the University of Illinois Herbarium, Mycological Collections No. 20849.

Growth on the original rabbit dung was very profuse, and other sporulating fungi were almost completely absent. Transfers to potato dextrose and to Blakeslee's No. 230 media were made with little difficulty and single spore cultures were subsequently made from these. This fungus grows more slowly on agar and with

much less cottony aerial mycelium than any isolate of *Choanephora* or of *Blakeslea* we have examined and such cultures are conspicuously zonate. In the dark zones, sporangiophores bearing sporangioles are formed in abundance but are very rare or completely lacking in the lighter zones. The relative abundance of zygospores in the lighter and darker bands is essentially uniform. Mature sporangioles are distinctly blacker than mature zygospores. The factor or factors controlling this growth pattern have not been completely determined but this seems to be influenced primarily by the effect of light on sporangiophore production. Walls of aerial hyphae, but especially those of sporangiophores, are conspicuously spotted with small hyaline crystals.

Sporangiophores arise from submerged hyphae, are relatively straight or somewhat curved and are terminated by a definite swelling (FIGS. 1, 2, 3, 9, 10, 11 and 12). Before this vesicle has enlarged very conspicuously, the initials of the sporangiole stalks appear. At first these are straight and the initial of the sporangiole itself soon arises as a swelling at the tip (FIGS. 2 and 10). During the period of enlargement of the sporangiole until it reaches almost its mature dimensions, very little elongation of the sporangiole stalk takes place (FIGS. 2, 10, and 11). At approximately the time a cross wall forms to delimit the sporangiole, the sporangiole stalk begins to elongate rapidly, recurving toward the vesicle (FIGS. 1, 3, and 12). Maturation of sporangioles appears to occur after the stalks have completed their elongation.

When sporangioles are crushed an interesting feature of this fungus can be observed that is otherwise likely to be overlooked. During the formation and maturation of spores, there is deposited against the sporangiole wall between the outer layer of spores thin bands of a darkly pigmented substance that sometimes remains attached to the wall to form a reticulation or in other instances may adhere to the layer of spores next to the wall (FIGS. 4 and 5). These delicate fibrils are readily separable from the spores or sporangiole wall on which they may be found when sporangioles are crushed in water, and so could not be regarded as part of the wall, either of spores or of sporangioles. This material appears rather to be clearly a residue from sporangiole protoplasm after spores have



FIGS. 8–12. *Cokeromyces recurvatus.*

been delimited. The spores themselves (FIG. 6) are smooth-walled with no trace of any striations or delicate radiating appendages at the ends, are not uniform in size and are quite variable in shape, usually ovoid to elliptical.

Zygospores (FIGS. 7 and 8) have been formed in great profusion on all types of culture media on which this fungus has been grown. Their abundance is comparable to that observed in cultures of *Zygorhynchus vuillemini* or of *Z. heterogamus* when grown on the same media. They usually form just above the surface of the culture medium, either between branches from hyphae that have just emerged, between branches from older more extensively developed hyphae, occasionally between branches of hyphae and branches from sporangiophores, or more frequently from branches from suspensors of more mature zygospores. They are in all respects much more like zygospores of *Cunninghamella* and of most of the genera of the Mucoraceae than like zygospores of *Choanephora* or of *Blakeslea* where the development of zygospores is more like that in species of the Cephalidaceae.

SUMMARY

A mucoraceous fungus belonging to the Choanephoraceae, representing a new genus and species, is described and named *Cokeromyces recurvatus*. The genus *Cokeromyces* is distinguished from *Blakeslea* and *Choanephora* by its more Mucor-like zygospores, its formation of sporangiola only as representing asexual reproductive structures, and by spores that lack radiating appendages and are not striated.

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URBANA

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EXPLANATION OF FIGURES

FIG. 1. Characteristic sporangiophore with numerous sporangioles. ×685. 2. Apical portion of young sporangiophore before much elongation of the sporangiole stalks has occurred. ×685. 3. Apical portion of small sporangiophore bearing only five sporangioles. ×685. 4. Crushed sporangiolum showing the net-like deposit on inner sporangiole wall which frequently becomes detached and clings to spores. ×1580. 5. Three of the outer layer of spores with associated detached deposit. ×2160. 6. Eight spores showing usual appearance. ×2160. 7. Two groups of zygospores in different stages of development. ×685. 8. Photomicrograph of portion of culture showing abundant zygospores in different stages of development and three sporangiophores as seen from above. ×275. 9. Photomicrograph of apical portion of very young sporangiophore. ×525. 10. Photomicrograph of slightly older condition than shown in figure 9. ×465. 11. Photomicrograph of young sporangioles on vesicle shortly before elongation of sporangiole stalks takes place. ×400. 12. Photomicrograph of apical portion of sporangiophore with mature sporangioles having strongly recurved stalks. ×250.

STUDIES ON A PLASMODIOPHORACEOUS PARASITE, OCTOMYXA BREVILEGNIAE

WILLIAM R. PENDERGRASS

(WITH 29 FIGURES)

The monotypic genus *Octomyxa* was established by Couch, Leitner, and Whiffen (**1939**). A second species of *Octomyxa* growing in *Brevilegnia linearis* was found in a soil collection from the City Park, Florence, S. C., in June, 1947. It appeared to be similar to *O. Achlyae* but because of negative growth reactions on *A. glomerata* and differences in the size of the galls, zoosporangia, and resting spores, it was believed to be a new species and given the name, *O. Brevilegniae* (Pendergrass, **1948**). A detailed study of this species has now been made.

MATERIALS AND METHODS

Hemp seeds were used as a culture medium throughout the study. The host and parasite were separated from other fungi by placing a mass of the parasitized hyphae of *Brevilegnia* on a 2 or 3 per cent plain agar plate, then separating the hyphae sufficiently to allow a hypha containing a parasitic plasmodium to be cut out on a block of agar. The agar block was then transferred to one of several large drops of water in a sterile Petri dish. The growth on the agar block was allowed to proceed for twelve to fifteen hours, after which a sterile split hemp seed was placed in the same drop of water in contact with the block. Efforts to grow *O. Brevilegniae* with its host on agar were unsuccessful.

Preliminary cytological studies were made using both whole mounts and sectioned material. As a result, it was found that the nuclei of the developing parasitic plasmodium undergo the "cruciform" or "protomitotic" type of division (FIG. 23). The heterocont, biflagellate nature of the zoospores (FIGS. 14, 26) was demonstrated by killing active zoospores in a drop of water on a slide

with fumes of 1% osmic acid and staining with gentian violet. The Löffler flagella staining technique (Couch, **1941**) was also used; but no tinsels were observed.

HOST RELATIONSHIPS

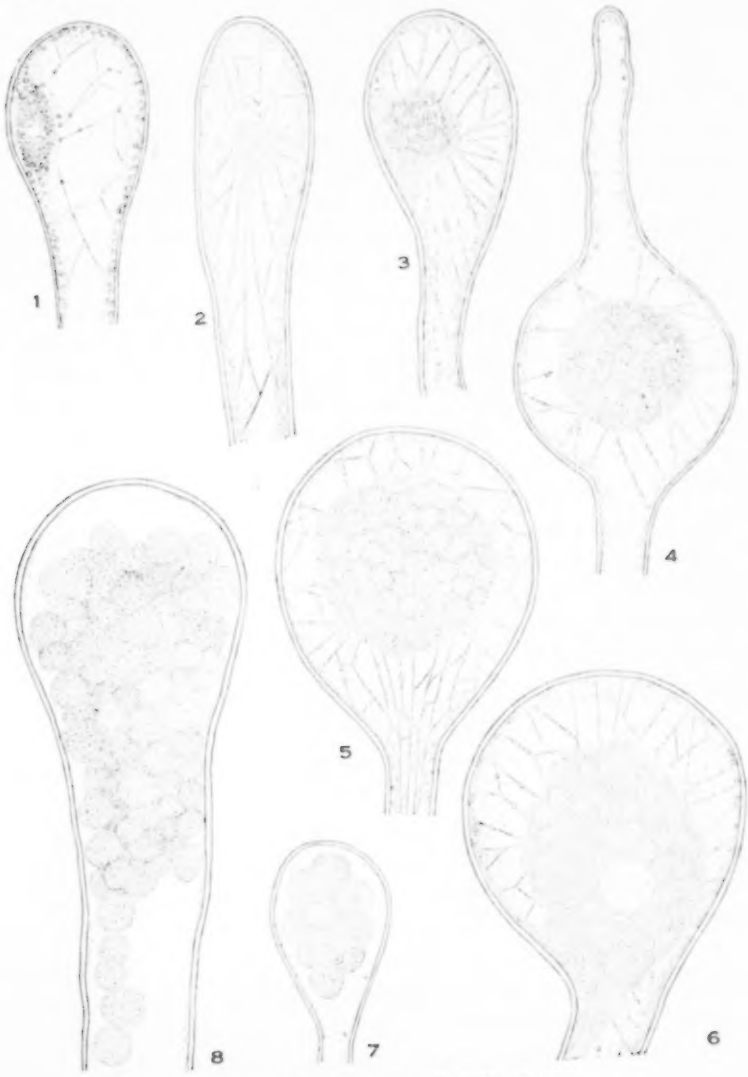
Numerous experiments to infect other water molds with *O. Brevilegniae* were carried out. Hyphae containing the parasite in various stages of development were placed in contact with cultures of various water molds likewise in different stages of growth. Hemp seed cultures of *Saprolegnia ferax, S. delica, S. diclina, S. litoralis, S. megasperma, Aplanes treleasanus, Dictyuchus missouriensis, Isoachlya intermedia, I. itoana, I. unispora, Aphanomyces laevis, Protoachlya hypogyna, Achlya racemosa, A. apiculata, A. colorata, A. caroliniana, A. glomerata, A. crenulata, A. flagellata, A. recurva, A. proliferoides, Thraustotheca primoachlya, T. unisperma, Allomyces javanicus, A. moniliformis, Apodachlya pyrifera* were found to be uninfected after association with the parasite, whereas *Geolegnia inflata* was as susceptible as *B. linearis*.

DEVELOPMENT OF O. BREVILEGNIAE

Zoospores of *O. Brevilegniae* have been seen on the hyphae of the host, but the actual penetration has not been observed even though considerable time was spent in search of such a process. However, it may be safely assumed that the entire zoospore passes into a hypha without first encysting because empty spore cases have never been observed on the hyphae which contained even the youngest stages of plasmodial development. In stained material, one quite frequently finds uninucleate plasmodia back some distance from the hyphal tips. The galls, however, almost always develop at the hyphal tips, carried there by the streaming protoplasm of the host.

This fungus produces two rather remarkable types of plasmodia. At the present time there is no thoroughly satisfactory explanation why one thallus of the parasite forms zoosporangia and another forms resting spores. The study of *Olpidiopsis* by Barrett (**1912**), however, offers the following possible explanation for this condition. The zoosporangia develop while the parasite is still young

and plenty of food material is available, whereas the resting spores develop as the host matures and while the food supply is being exhausted. Accordingly, the parasite is provided with facilities for rapid propagation as well as survival during adverse conditions.



FIGS. 1–8. *Octomyxa Brevilegniae.*

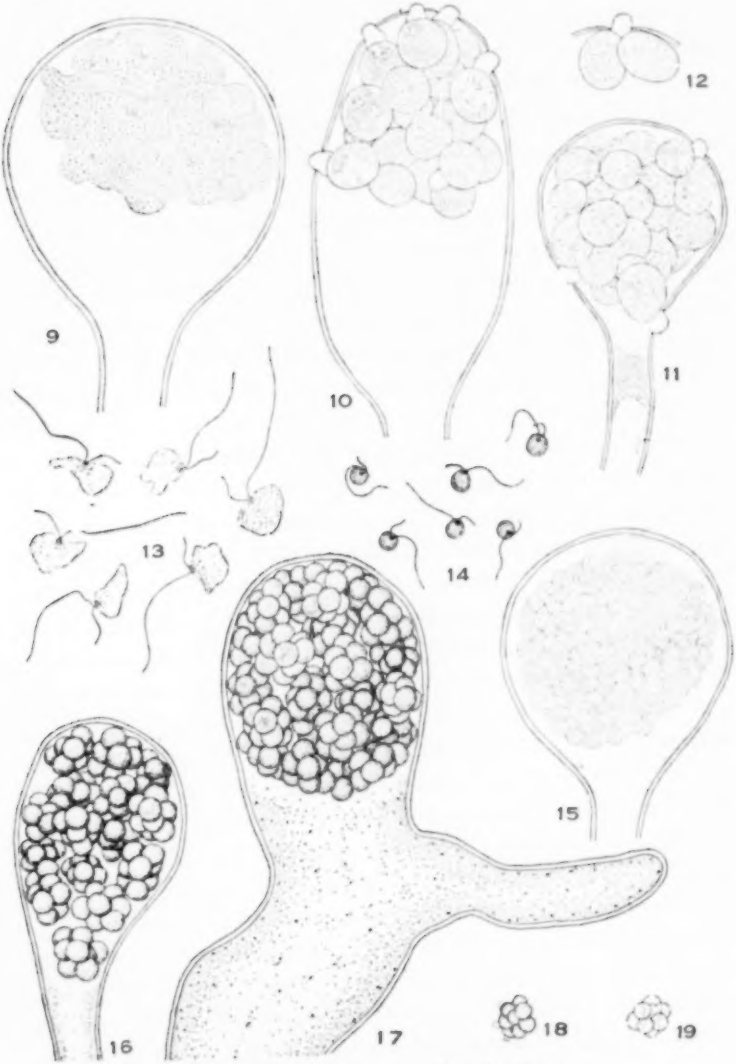
If a new culture is made by cutting off infected hyphae from an old culture and placing them in contact with a piece of hemp seed, new infections of the host will appear in about twenty to thirty hours. Heavily granular hyphae which show a slight swelling at the tips or at a position just behind the tips begin to appear close to the hemp seed. These swellings assume the typical spherical shape after another day of growth. As new infections continue, fully 65 per cent of the host hyphae may show indications of the parasite but later-formed galls are somewhat smaller than the first. Several galls have been seen in each of which there was more than one developing plasmodium. Yet, each plasmodium was clearly independent of the others with no fusion occurring among them.

The study of living plasmodia was made with both water immersion and oil immersion objectives. It was found that the parasite in its early stages takes a position close to the hyphal wall near the tip (FIGS. 1, 21). In this position, there is a flow of globules toward the parasite along cytoplasmic threads of the host, with the result that there is a conspicuous accumulation of these globules around the plasmodium. The globules later become enclosed within the plasmodium. Therefore, it seems obvious that the globules along with the host protoplasm are being drawn into the plasmodium as food. Host protoplasm continues to be drawn into the plasmodium from the expanded hyphal tip as well as from that region of the hypha below the gall. Consequently, the unwalled parasite increases in size (FIG. 3) and soon takes up a position in the center of the gall (FIGS. 4, 22). At the same time, the plasmodium assumes an uneven, reticulate appearance such that lighter areas unequal in size become faintly visible within the rounded mass (FIGS. 5, 24).

Further enlargement of the parasite takes place with the gradual exhaustion of the available host protoplasm. Concurrently with this increase in size, vacuoles may appear within the plasmodium (FIG. 6).

The first evidence of zoosporangial formation is the cleaving of the parasitic thallus into numerous rounded masses which as development proceeds gradually become more distinct (FIGS. 7, 8). Each of these spherical masses becomes a zoosporangium. At this

time, the young emergence papillae directed toward the host wall may be noticed on some of the zoosporangia. These papillae contain granules at first but become hyaline, the granules apparently flowing back into the body of the sporangium (FIGS. 9, 10, 11).



FIGS. 9–19. *Octomyxa Brevilegniae.*

Cleavage of the sporangial protoplasm begins as a slight furrowing from the center outward. The spore initials thus have a rounded appearance toward the center of the sporangium with the opposite side conforming with the curvature of the periphery of the sporangium (FIG. 12).

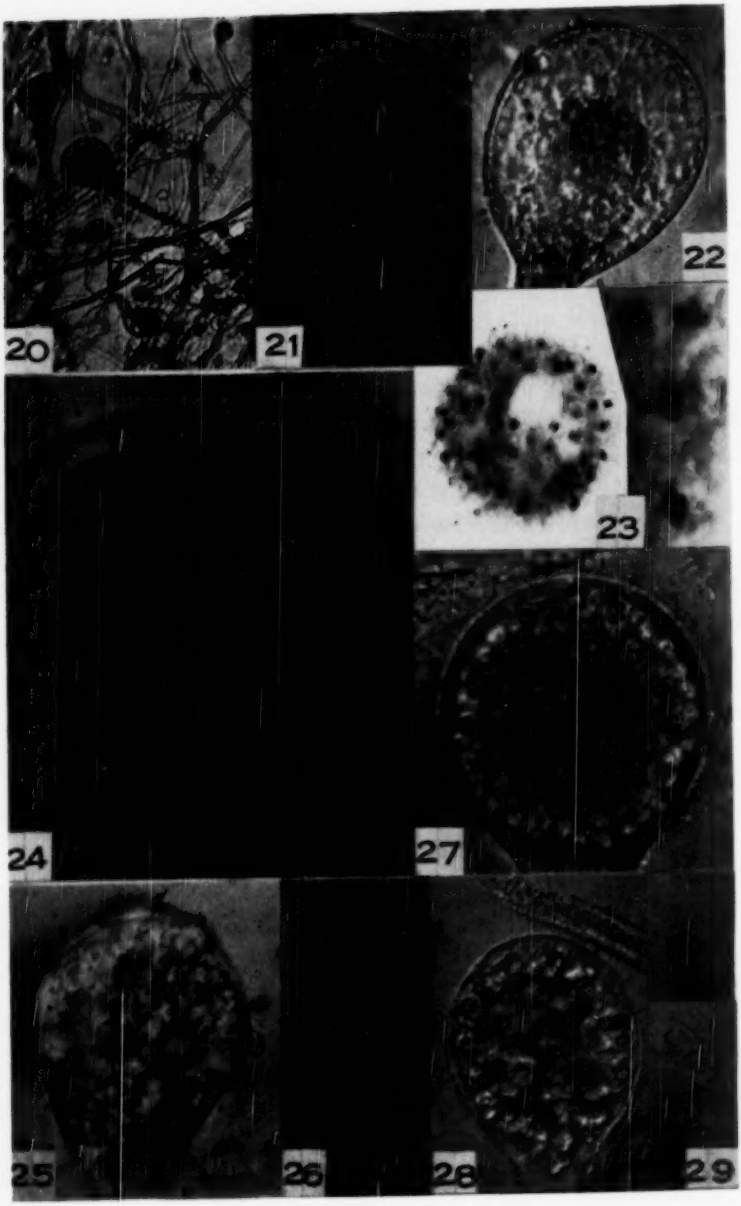
Not all the zoosporangia in any given sorus mature simultaneously. Only the peripheral zoosporangia have papillae. Those situated deeper in the gall are connected to one another and subsequently to the peripheral sporangia by coinciding apertures in their closely appressed sporangial walls. The exit papillae soon penetrate the hyphal walls and finally rupture. Thus the zoospores as they mature have a passageway to the outside. It is not at all uncommon that an exit tube may appear at the juncture of two peripheral sporangia, thus forming the emergence-pore for these two as well as the sporangia beneath them (FIGS. 12, 25). The writer, on several occasions, has seen an exit papilla which gave the appearance of occurring at the juncture of three and even four sporangia. The zoosporangial walls persist after zoospore discharge, disintegrating only after the hyphae of the host have broken down. They give a negative reaction for cellulose.

Flagella are formed on the spores while they are still in the sporangia and considerable movement may be noted prior to their escape. After the sporangia become partly empty, some spores may move from one sporangium to another and then back to the sporangium in which they were formed. The first spores to emerge from the exit papillae appear to be thrust out very violently whereas later ones escape much more slowly. The spores themselves have a peculiar amoeboid posterior end which may bear one or two delicate protoplasmic processes (FIG. 13). After the body of the spore has passed through the exit pore, these delicate posterior processes usually become hung in the papilla whereupon the spore frees itself only after a violent struggle.

Swimming actions of the spores were quite difficult to follow. Some spores exhibited either a rocking motion or a trembling movement whereas others swam in a nearly straight path rotating clockwise on their axes, as viewed under the microscope, with the long posterior flagellum being much less active than the short anterior one.

The development of resting spores takes place in plasmodia very similar to those from which zoosporangia are formed, but it is not easy to distinguish between the two. However, the plasmodia which form resting spores are noticeably darker, due to their much heavier granular contents. Spore formation begins as a rounding up of the unwalled plasmodium into masses which approximate the size of the future resting spores (FIGS. 15, 27). These future spores are rather hard to distinguish and can best be seen on the periphery of the plasmodium as a roughened border. Gradually, the roundness of the spore initials becomes more evident until finally individuals are distinguishable. At this time, there is no indication of an octant grouping and the initials themselves are without definite walls. Once the spores become mature, the groups of eight can be readily seen (FIG. 16). Rarely one finds groups containing seven or nine spores. Although there is no membrane or wall around the groups of eight spores, each group remains intact even after the host wall has disintegrated and the groups are floating in the water.

In view of the importance of determining the ultimate fate of the resting spores, efforts were made to stimulate their germination. Preliminary endeavors consisted simply of cutting from the hemp seed several hyphae containing an appreciable number of cystosori galls, placing the hyphae in a large drop of sterile water on a slide and housing the slide in a damp chamber. Observations were made twice a day for seven days but no indications of germination were noted. Alternate wetting and drying which Jahn (**1905**) found important in the germination of the spores of certain Myxomycetes proved fruitless with the resting spores of *O. Brevilegniae*. In addition, spore galls were subjected first to a temperature of 36° C. for two days, then to a temperature of 0° C. for an equal length of time and vice versa for a second group of galls, but again results in both cases were negative. Further germination studies were made by placing cultures of *B. lincaris*, which contained resting spores, into watch glasses, some of which were filled with sterile charcoal water and others with spring water. The watch glasses were then placed in a cabinet with the temperature maintained at 23° C. to 28° C. Nevertheless, the resting spores failed to germinate. Finally, resting spore galls were treated with a number of



FIGS. 20–29. *Octomyxa Brevilegniae.*

decoctions. The first of these decoctions was made by boiling about 50 unparasitized cultures of *B. linearis* in 60 cc. of distilled water for 15 minutes after which the liquid was filtered and sterilized. Drops of the filtrate were placed on a series of slides to which spore galls varying in age from 2 weeks to 2 months were added. Examination, however, showed only negative results for all ages of spores treated. As for the other decoctions, 20 grams of lima bean seedlings (roots and old cotyledons removed), old leaves of rock chestnut oak, new leaves of southern red oak, crushed corn seeds, pine needles, and crushed green peas were each boiled in 60 cc. of distilled water for 15 minutes, filtered, and sterilized. Spores were placed in filtrates of each of the six above-mentioned decoctions as well as in filtrates which were diluted with distilled water in ratios of 3 : 1, 1 : 1, and 1 : 3. The pH of these decoctions ranged from 4.1 for the pine needles to 7.7 for the green pea decoction. No germinations were obtained in any of these decoctions.

Although the actual germination of the resting spores has not been observed, many of the groups of eight spores have been seen upon which wart-like protrusions had formed (FIG. 18). Proof that the resting spores do germinate was supported by the finding of many octads of empty spore cases each with a delicate pore in its wall (FIG. 19). Further circumstantial evidence of germination was obtained from cultures. New growths of the parasite on *B. linearis* were obtained on hemp seeds which had been placed in contact with old (94 days) infected cultures of the host following a change to Petri dishes with fresh water. Consequently, it would appear that spore germination took place and that a period of aging, such as Ziegler (1948) found necessary for zygote germination in members of the Saprolegniaceae, is necessary in order to obtain positive results.

SUMMARY

O. Brevilegniae was found to be an obligate parasite on *Brevilegnia linearis* and *Geolegnia inflata*. Infection of the host hypha is by a zoospore. No spore case is left on the hypha. All regions of the hyphae are susceptible to penetration, but the galls are predominantly formed at the hyphal tips. Increase in size of the

vegetative thallus is correlated with a reduction in quantity of the host protoplasm in the infected hypha. The plasmodium which is to form zoosporangia rounds up into homogeneous masses. Cleavage of spores from these sporangial initials is from the center outward. Only the peripheral zoosporangia have exit papillae. Those situated deeper in the gall are connected to one another by coinciding apertures in their walls and subsequently to those with papillae. One papilla may appear at the juncture of two or perhaps even three or four peripheral sporangia. Motile zoospores have an amoeboid posterior end as well as a short anterior flagellum and a long posterior flagellum which ends in a whip lash. Resting spores are derived from plasmodia similar to those from which zoosporangia originate, but the former plasmodia are darker in appearance than the latter. These plasmodia are cleaved predominantly into groups of eight spores. Germination of the resting spores seems to be dependent upon an aging period. The empty spore case showed a delicate pore through which the spore emerged.

ACKNOWLEDGMENTS

The writer is grateful to Dr. J. N. Couch for guidance during this study, to Mrs. Alma Beers for arranging the figures, to A. W. Ziegler for cultures of water molds, and to C. D. Beers for information concerning "protomitosis" in the Protozoa.

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EXPLANATION OF FIGURES

All drawings approximately × 735 except figure 23

FIGS. 1–4. Early stages of parasite development. FIG. 5. Plasmodium with reticulate appearance. FIG. 6. Plasmodium with vacuole. FIGS. 7, 8. Young zoosporangia. FIG. 9. Emergence papillae forming. FIGS. 10, 11. Cleavage of spores. FIG. 12. Exit papilla shared by two sporangia. FIG. 13. Sketch of active zoospores. FIG. 14. Stained zoospores with whip lash. FIG. 15. Cleavage of resting spores. FIGS. 16, 17. Resting spore galls. FIG. 18. Octad of resting spores, three of which are excysting. FIG. 19. Empty cysts with pores. FIG. 20. Several galls of *Octomyxa*, × 75. FIG. 21. Parasite close to hyphal wall, × 755. FIG. 22. Plasmodium in center of gall, × 575. FIG. 23. Plasmodium stained with Gram's gentian violet technique. Small photograph of entire plasmodium at left, × 690. Three nuclei in "Protomitotic" division enlarged from original photograph, × 2770. FIG. 24. Zoosporangial plasmodium mature in size, × 755. FIG. 25. Zoosporangial gall showing exit papilla, × 755. FIG. 26. Zoospores stained with Löffler technique, × 755. FIG. 27. Cleavage of resting spores, × 755. FIG. 28. Resting spore gall, × 575. FIG. 29. Groups of free resting spores, × 575.

TWO NEW SPECIES OF HIRSUTELLA PATOUILLARD

FRAN. E. FISHER

(WITH 4 FIGURES)

During the summer and fall of 1948, epizootics were observed of two economically important arthropods, rust mites [*Phyllocoptruta oleivora* (Ashm.)] and purple scales (*Lepidosaphes beckii* Newm.), on *Citrus* in Florida. The occurrence of a fungus consistently associated with dead first instar nymphs of purple scales was reported by Fisher *et al.* (2), and tentatively identified as a species of *Hirsutella*. Later, a fungus on dead rust mites was also reported (1) as probably being *Hirsutella* sp. Further study has shown that these fungi on dead rust mites and dead first instar nymphs of purple scales are not only separate and distinct species, but also are species which have not previously been described.

Both of these fungi are easily recognized under a dissecting microscope with a magnification of 15 ×. On young purple-scale nymphs, the *Hirsutella* exhibits an extensive growth of mycelium which originates from the ventral surface of the insect's body. Only a few hyphae are present on rust mites; these hyphae typically extend posteriorly or anteriorly from the mite's body, but, occasionally, lateral hyphae are found. The direction of the mycelial growth depends upon the portion of the mite's body from which the hyphae emerge. For instance, if a hypha emerges from the posterior region of a rust mite's body, the typical growth of that hypha will be a direct extension posteriorly from the body.

Since the larger-bodied scales give rise to a fuller mycelium than do the smaller-bodied mites, the variations between the two species were at first considered to be non-specific and to be directly related to the food supply. It has been found, however, that the dissimilarities between these two species are not only in size and extent of mycelium, but also in the shape of the phialides and the spores, and the color of the older mycelia.

Morphologically, neither of these species of *Hirsutella* is entirely typical of the genus. Although the conidiophores are characteristic of *Hirsutella*, the production of synnemata has not been observed on rust mites and only two synnemata have been found on young purple-scale nymphs. Other workers have observed that when growing with an insufficient supply of food often the synnemata are not produced, but, instead, single hyphae. Therefore, it would appear that these species do belong to the genus, *Hirsutella*, but that they grow on such small hosts that they can not, or only infrequently, produce synnemata.

Each of these species exhibits a specificity as to host. The species which attacks first-instar purple scales has not been found on rust mites and *vice versa*. Neither of these species has been observed growing on any other insects which are found on *Citrus*.

Whether or not these fungi are responsible for the periodic epizootics of young purple scales and rust mites remains to be proved. However, if they are not parasitic they both appear to be specific indicators of one particular disease of each of the two arthropods.

Hirsutella Besseyi sp. nov. on LEPIDOSAPHES BECKII Newm.

Entomogena. Synnematibus plerumque deficientibus sed raro praesentibus. Mycelio primo albo, deinde cinereo vel brunneo; hyphis erectis vel procumbentibus, 3.3–5.8 μ diam., septatis; conidiophoris a latere vel erectis, rigidis, attenuatis, supra hyalinis infra leve fuliginis, 29.9–74.7 μ longe; phialidibus acuminatis 2.5–4.9 $\mu \times$ 21.6–66.4 μ, typice in sterigmata attenuatis, 8.3–19.9 μ longe, aliquando 1–3 additicia sterigmata minus 8.3 μ ab phialidis apici gignentibus; conidiis ad apicem, hyalinis, anguste elongatis sed citriformis a capsula gelatinosa visis, 2.5–5.8 $\mu \times$ 4.1–8.3 μ. Rhizoidibus digitatis.

Hab. in nymphorum primo instar *Lepidosaphes beckii*, in foliis *Citri* spp. prope Lake Alfred, Florida, Amer. Bor. Aug., 1948, Febr., 1949, et Mart., 1949.

Entomogenous. Synnemata infrequently produced. Mycelium white, later gray to brown; hyphae erect or decumbent, 3.3–5.8 μ in diameter, septate; conidiophores lateral or erect, rigid, tapering, hyaline above, pale fuliginous below, 29.9–74.7 μ in length; phialides acuminate, 2.5–4.9 $\mu \times$ 21.6–66.4 μ, typically attenuated into a sterigma 8.3–19.9 μ in length, occasionally 1–3 additional sterigmata developing within 8.3 μ of phialidal apex; conidia apical, hyaline, narrowly elongate but appearing lemon-shaped due to a gelatinous capsule, 2.5–5.8 $\mu \times$ 4.1–8.3 μ. Rhizoids digitate.

On first instar nymphs of *Lepidosaphes beckii* Newm., on leaves of *Citrus* spp., vicinity of Lake Alfred, Florida. August, 1948, February and March, 1949.

It has been observed by the author that occasionally large numbers of first-instar purple scales, which are typically white in color,

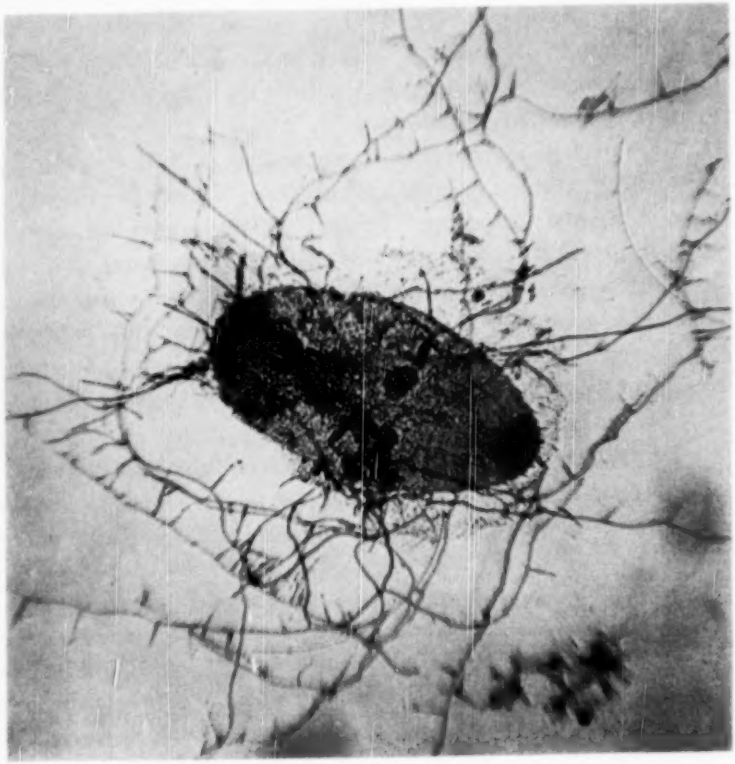

FIG. 1. *Hirsutella Besseyi.*

become yellow and a high mortality follows. A species of *Hirsutella* has been found to be consistently associated with nymphs exhibiting the abnormal yellow color.

The mycelium was well developed over the dorsal surface of the host, and was attached to the leaf surface by digitate rhizoids. With a hand lens the fungus appeared translucent to silvery white in color. Older specimens appeared gray to brown.

In early stages the mycelium was fine, hyaline, and septate, later developing thicker cell walls and becoming pale fuliginous in color. Hyphae occurred singly, averaging 4.0 μ in diameter, ranging from 3.3 μ to 5.8 μ. Closely associated parallel hyphae have not been frequently observed. Typically, synnemata were not produced, but in two instances have been found. Figure 1 shows a first-instar nymph of purple scale with a well-developed mycelium bearing conidiophores and conidia.

The fungus fruited abundantly. The conidiophores were lateral or erect and produced regularly on the mycelium. The phialides were acuminate in shape and attenuated into a long sterigma. Occasionally one to three additional sterigmata occurred within 8.3 μ of the apex of the phialide. Phialides bearing more than one sterigma were more commonly observed in older specimens than in young specimens. Each sterigma bore a single, narrowly elongated spore which was surrounded by a gelatinous matrix, or capsule, thereby giving the spore the appearance of being lemon-shaped. Figure 2 shows two conidiophores each of which is attenuated into one sterigma bearing a conidium.

In naming this species, it is the desire of the author to honor a great mycologist, teacher, and friend, Ernst A. Bessey.

Hirsutella Thompsonii sp. nov. on PHYLLOCOPTRUTA OLEIVORA (Ashm.)

Entomogena. Synnematibus deficientibus. Mycelio primo albo deinde griseo; hyphis procumbentibus, 1.7–3.3 μ in diam., breve septatis; conidiophoris a latere vel erectis, rigidis, hyalinis, 10.8–16.7 μ longe; phialidibus conicis ad strobiloidibus, 3.3–4.9 × 5.4–9.9 μ, typice in dua sterigmata attenuatis, 1.7–6.7 μ, saepe uno sterigmato vel aliquando tribus sterigmatis apice gignentibus; conidiis apicibus, hyalinis, ovoidis vel rotundis, citriformis a capsula gelatinosa visis, 2.1–3.3 × 2.1–3.3 μ. Rhizoidibus digitatis.

Hab. in *Phyllocoptruta oleivora* (Ashm.) in foliis *Citri* spp. prope Lake Alfred, Florida, Amer. Bor. Aug., 1948, Febr., 1949, et Mart., 1949.

Entomogenous. Synnemata not produced. Mycelium white, later grayish; hyphae decumbent, 1.7–3.3 μ in diameter, short-septate; conidiophores lateral or erect, rigid, hyaline, 10.8–16.7 μ in length; phialides conoid to strobiloid, 3.3–4.9 × 5.4–9.9 μ, typically attenuated into two sterigmata, 1.7–6.7 μ, but frequently only one sterigma or occasionally 3 sterigmata develop at apex;

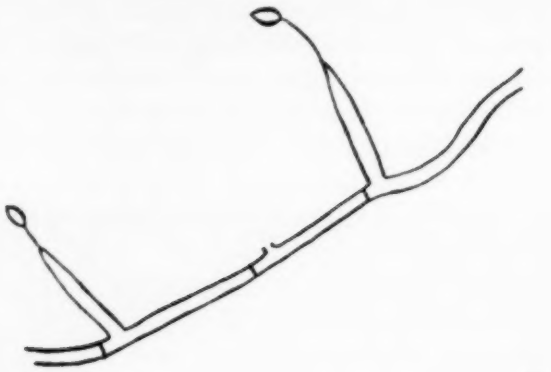

FIG. 2. Mycelium, phialides, and spores of *Hirsutella Besseyi*. 645 ×.
FIG. 4. Mycelium, phialides, and spores of *Hirsutella Thompsonii*. 645 ×.

conidia apical, hyaline, oval to round, may appear lemon-shaped due to a gelatinous capsule, 2.1–3.3 × 2.1–3.3 μ. Rhizoids digitate.

On *Phyllocoptruta oleivora* (Ashm.) on leaves of *Citrus* spp., vicinity of Lake Alfred, Florida. August, 1948, February and March, 1949.

Type specimens of *Hirsutella Besseyii* and *H. Thompsonii* have been deposited in the Herbarium of the University of Florida, Gainesville, Florida, the Farlow Herbarium, at Harvard University, Cambridge, Massachusetts, and the Herbarium of the Commonwealth Mycological Institute, Kew, Surrey, England.

During October, 1948, an epizootic of rust mites occurred in an unsprayed control plot at Lucerne Park, Florida. Large numbers of the living mites exhibited a dark yellow to tan color rather than the light lemon-yellow color which is typical of healthy rust mites. The characteristics of these abnormal mites correspond to the description by Speare and Yothers (**3**). Following the occurrence

of these large numbers of off-colored mites, a high mortality resulted in the rust mite population. In association with the death of the mites a fungus identified (1) as a species of *Hirsutella* was consistently found to be present on the mites.

Mites which had been dead for a short time and which exhibited a dark yellow to light brown color were mounted for examination.

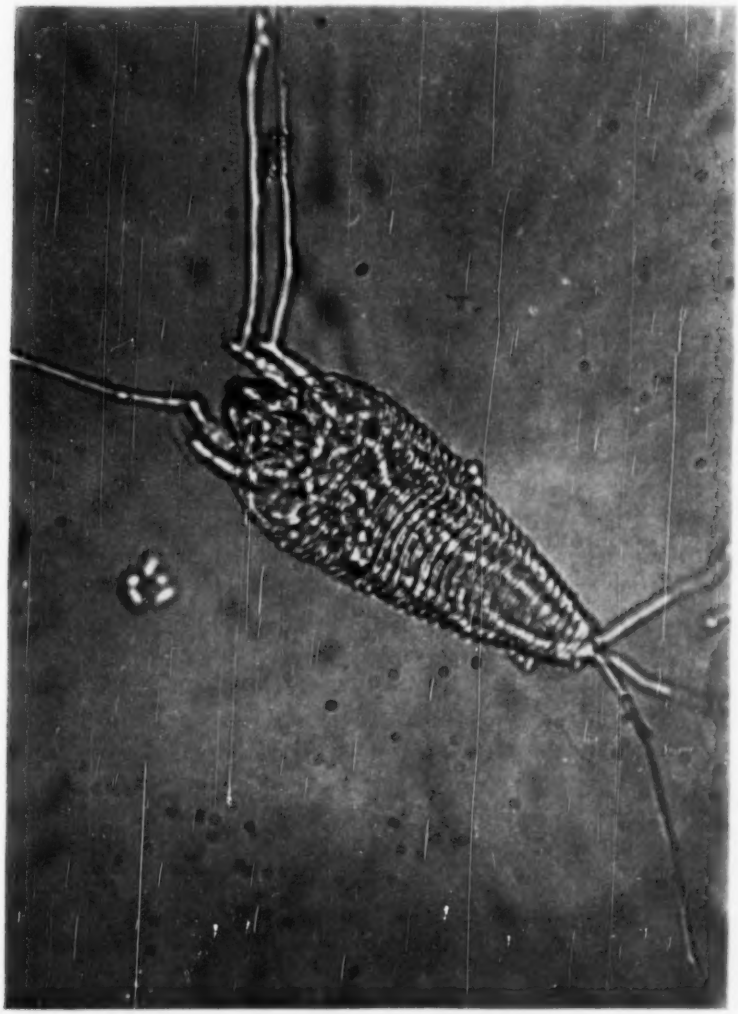

FIG. 3. *Hirsutella Thompsonii.*

Some of these mites contained one or more strands of a fungus mycelium growing longitudinally through the body. On mites which had been dead for a longer period of time, hyphae were growing apparently from the interior through both the anterior and posterior portions of the body. In some specimens lateral emergence of the hyphae occurred. The anterior and posterior emergence of 1–4 hyphae was typical. Under a hand lens, the fungus was silvery-white in color.

During all stages of growth the mycelium was fine and hyaline. The mature hyphae were septate. Hyphae averaged 2.4 μ in diameter, but ranged from 1.7 μ to 3.3 μ. All of the hyphae occurred singly, did not adhere laterally in synnemata, and were attached to the leaves by digitate rhizoids. Figure 3 shows a rust mite with 3 young hyphae growing from both the anterior and the posterior regions. The three hyphae growing from the anterior portion of the mite's body emerged through three of the mite's legs. Several hyphae can be seen extending longitudinally through the body of the mite.

The fungus fruited rather abundantly. Conidiophores were lateral or erect and were produced regularly on the mycelium. Phialides were conoid to strobiloid and attenuated into one or more sterigmata. Although one sterigma was frequently produced by each phialide, the phialides seemed to be typically terminated by two sterigmata, and occasionally three sterigmata were observed on one phialide. Each sterigma bore one oval or round spore surrounded by a gelatinous matrix, or capsule, which in some cases gave the spore a lemon-shaped appearance. Figure 4 shows mycelium bearing three phialides one of which bears two sterigmata.

This species is named to honor a colleague, W. L. Thompson, who has been instrumental in developing the spraying schedule for *Citrus* in Florida, and who provided the author with the first specimens.

ACKNOWLEDGMENTS

The author is indebted to Mr. Erdman West, Botanist and Mycologist, Florida Agricultural Experiment Station, Gainesville, Florida, for the preparation of the Latin diagnoses; to Dr. Ernst A. Bessey, Professor of Botany, retired, Michigan State College,

East Lansing, Michigan, for assistance in the identification of the species herein identified; and to Dr. L. Carl Knorr, Histologist, Citrus Experiment Station, Lake Alfred, Florida, for helpful suggestions in the preparation of the manuscript.

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A MEDIUM FOR THE GROWTH AND MAINTENANCE OF THE YEAST-LIKE PHASE OF HISTOPLASMA CAPSULATUM

E. H. Titsworth and E. Grunberg *

(with 1 figure)

The production and maintenance of the yeast-like phase of *Histoplasma capsulatum* is known to be difficult. Numerous media have been recommended such as blood agar (Conant, **1941**) and Francis' cystine glucose agar (Campbell, **1947**). These solid agar modifications have, however, a disadvantage in that transfers must be made at short intervals and the yeast-like phase is not obtained consistently.

During recent experiments, in an attempt to obtain the yeast-like phase of *H. capsulatum*, the egg-yolk-potato medium commonly used for the growth of *Mycobacterium tuberculosis* was found to be of value. Twenty-three strains of *H. capsulatum* which were obtained from Vanderbilt University were found to develop a yeast-like phase on this medium within one to two transfers if grown at 37° C. These cultures have maintained their yeast-like phase on repeated subculture for a minimum period of thirty days.

The formula and preparation of the egg-yolk-potato medium (Sasano and Medlar, **1943**) is as follows:

A. Potato base

Potato—peeled and finely ground	200	gm.
Glycerol	60	ml.
Citric acid	0.2	gm.
Bacto-hemoglobin	5	gm.
H_2O	1000	ml.

* From the Chemotherapy Laboratories, Hoffmann-La Roche Inc., Nutley, N. J.

Autoclave at 15 lbs. pressure for 30 minutes. Filter through a double layer of gauze. Flask in 500 ml. quantities and autoclave at 15 lbs. pressure for 30 minutes.

B. Egg-yolk mixture

Clean eggs, soak in 80 per cent alcohol for 2 hours. Decant alcohol and dry eggs by flaming. Separate whites from yolks into a sterile graduate. A proportion of one whole egg to eleven egg yolks is used. Filter egg plus egg-yolk through a double layer of sterile gauze.

FIG. 1. Yeast-like growth of *Histoplasma capsulatum*.

Mix 500 ml. of the sterile egg-yolk filtrate with 500 ml. of sterile potato-base. Add 10 ml. of sterile 10 per cent congo red. Dispense the mixture into sterile test tubes or Petri dishes as desired and inspissate in free-flowing steam in the autoclave with no pressure for one hour. Incubate for sterility.

All test tubes or Petri dishes should be kept well sealed with paraffin or parafilm to preserve moisture.

An example of the type of yeast-like growth obtained on first or second transfer from the mycelial phase is depicted in figure 1.

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NO BRACHYMEIOSIS IN PYRONEMA CONFLUENS

HILDE E. HIRSCH

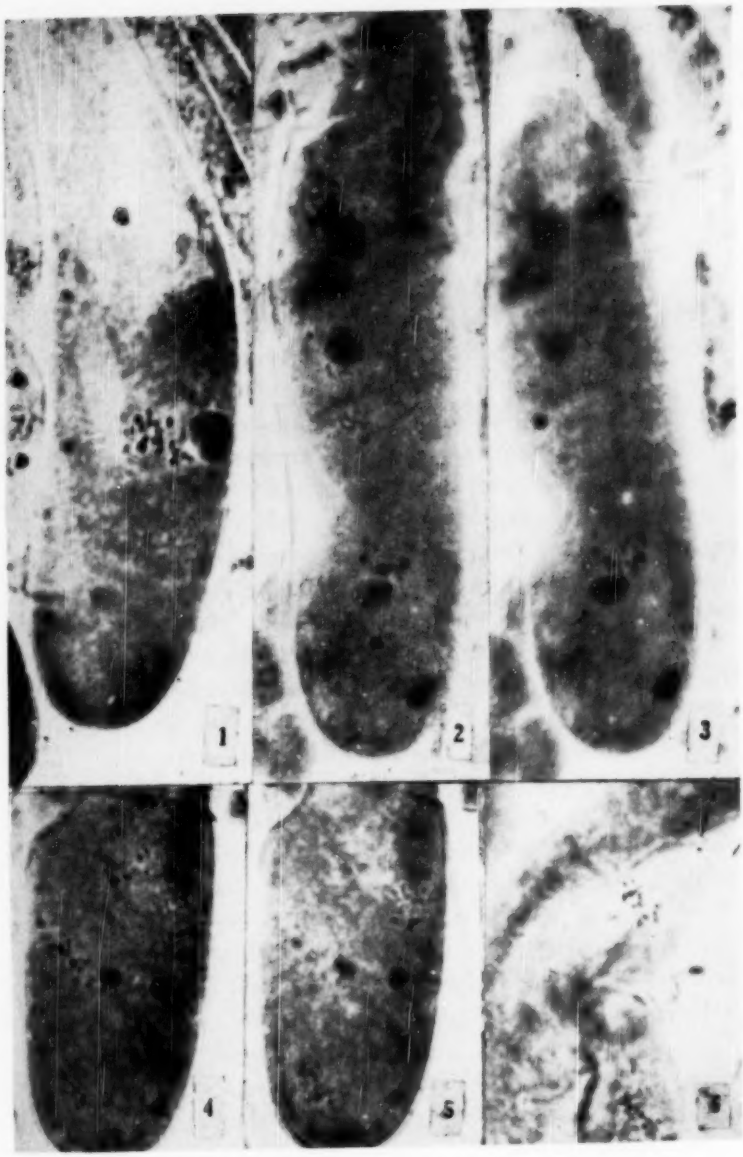
(WITH 6 FIGURES)

The question whether two reduction divisions occur in the life cycle of at least some ascomycetes is one which was argued vehemently for over 30 years. Swingle (**1934**) has given an excellent summary of the numerous reports supporting or denying the occurrence of two nuclear fusions (one in the ascogonium and one in the ascus), and their necessary consequence, double chromosome reduction or "brachymeiosis." The organism which has played a central role in these investigations and discussions is the well known *Pyronema confluens* Tul. [*P. omphalodes* (Bull.) Fuckel].

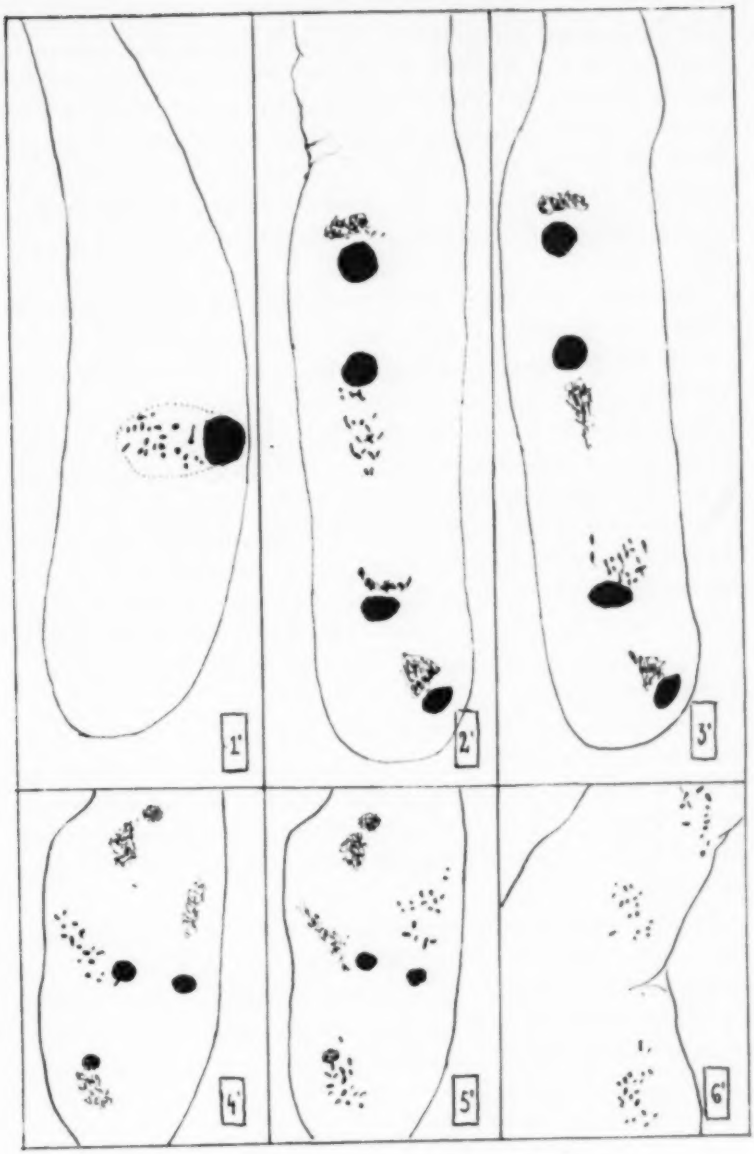
More recently, the genetical and cytological work done on a number of ascomycetes has served to strengthen the view that brachymeiosis does not occur. The present brief note is intended to present some photomicrographs of preparations obtained with the acetocarmine smear technique which, in the opinion of the writer, show that in the classical *P. confluens* the claims of two successive reductions in chromosome number are erroneous.

Materials and methods: The strain of *P. confluens* was obtained through the courtesy of Dr. H. N. Hansen. It is the common form and not identical with Brown's var. *inigneum* which is able to grow on unheated substrates and is apogamous (Brown, **1915**). The fungus was grown on Hansen and Snyder's medium (**1947**), consisting of plain agar mixed with pieces of pea straw which have been sterilized by propylene oxide. In the diffuse light outside a north window, it produced apothecia readily and in large quantities. They were fixed in a solution consisting of 1 part glacial acetic acid saturated with iron acetate (Cave and Rattenbury, unpublished), 2 parts absolute alcohol and 3 parts ether, and stained with iron-acetocarmine.

Description of photomicrographs: Figure 1 shows an early anaphase of the first meiotic division in the ascus. 24 Chromosomes



FIGS. 1–6. Division figures in *Pyronema*.



FIGS. 1'–6'. Drawings of figs. 1–6.

are visible, 12 (bivalents) going to each pole. Figures 2 and 3 show the anaphase of the third division in two of the four visible nuclei in one ascus. Similarly, in another ascus, figure 4 shows an early anaphase of the third division in one nucleus, and figure 5 in two other nuclei. Figure 6, finally, shows three third division nuclei in still another ascus. All the nuclei seen in figures 2–5 contain more than 12 chromosomes (although not all 24 can be counted in any one photograph), indicating that more than 6 will pass to each pole.

Discussion: According to Gwynne-Vaughan and Williamson (**1931**), the number of chromosomes in the germinating spore, the cells of the apothecial sheath, paraphyses, etc., is 6 (the haploid number); in the ascogenous hyphae it is 12, and in the definitive ascus nucleus there are 12 gemini (4n). In the third division in the ascus, according to these authors, the number is again reduced to 6. Our observations, on the contrary, confirm the reports of Claussen (**1912**) and of Moreau and Moreau (**1931**), which state that 12 chromosomes go to each pole in the third division, the haploid number being 12. The writer has not been able to count chromosomes in either the somatic cells or the ascogenous hyphae, but in the conjugate division in the crozier, approximately 12 chromosomes were seen.

No attempt was made to observe the behavior of the male and female nuclei in the ascogonium after fertilization, though the movement of the antheridial nuclei through the trichogyne was seen. However, if no brachymeiosis occurs in the ascus, as we believe to have shown, no nuclear fusion in the ascogonium would be expected.

The argument remains that different strains of the fungus may differ with regard to their nuclear behavior. Unfortunately, the original strain of Gwynne-Vaughan and Williamson appears to have been lost, but the strain occurring in Britain should also be reexamined with the modern smear technique.[1]

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[1] Olive's re-investigation of *Patella melaloma* (Science **110**: 185 (1949)), published after the above note went to press, has recently shown that in that fungus, too, the claim of brachymeiosis was erroneous.

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EXPLANATION OF FIGURES

FIG. 1. First division of the definitive ascus nucleus, early anaphase. 12 chromosomes (bivalents) moving to each pole. FIG. 2. Anaphase of third division, four nuclei are visible. In the third nucleus from the tip the chromosomes can be counted. FIG. 3. The same ascus as in figure 2, focused to show the chromosomes in the second nucleus from the tip. FIG. 4. Another four-nucleate ascus. Chromosomes can be counted in the left middle nucleus. FIG. 5. The same ascus as in figure 4, focused to show the chromosomes in two other nuclei. FIG. 6. Third anaphase in another ascus. Three nuclei visible. All figures about 1750 ×.

THE GENUS GIBELLULA ON SPIDERS IN NORTH AMERICA [1]

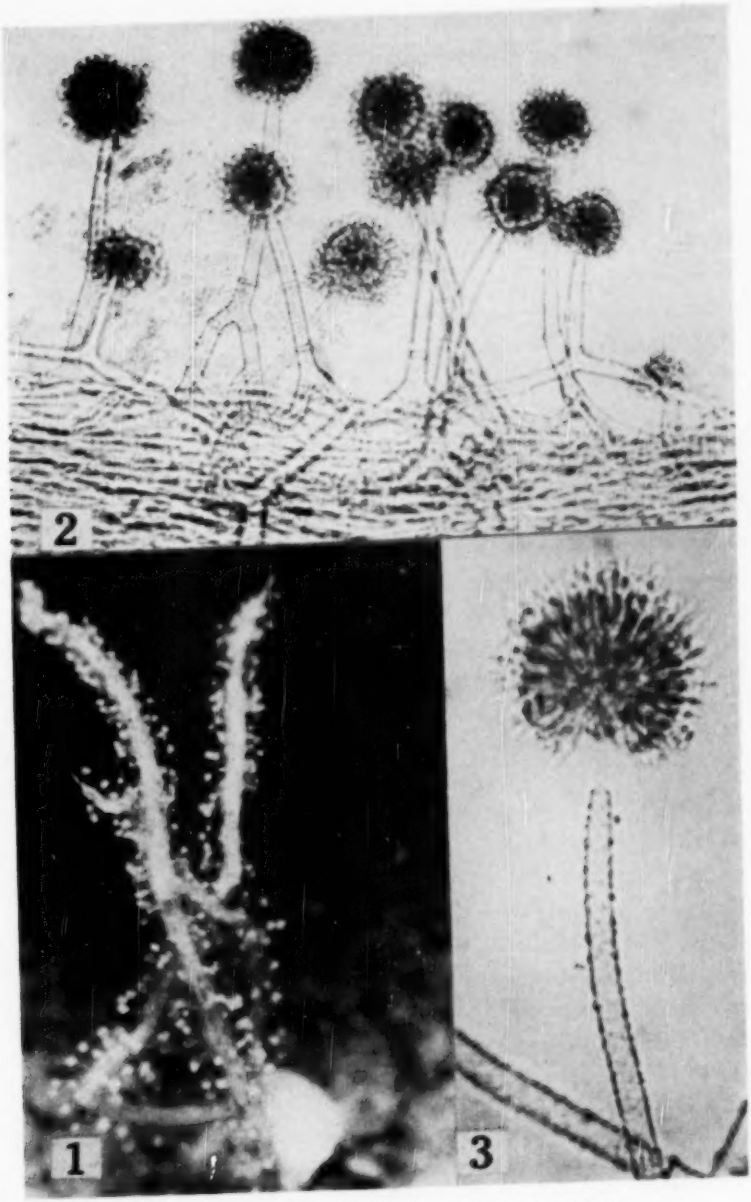
E. B. Mains

(WITH 14 FIGURES)

In 1894 the genus *Gibellula* was proposed by Cavara (**4**) based on *Gibellula pulchra* (*Corethropsis pulchra* Sacc.). Since then a number of species have been described mostly on spiders. In 1932 Petch (**18**) published a paper concerning the genus, placing most of the names in synonymy and recognizing only two species, *G. aranearum* (Schw.) Syd. and *G. alata* Petch. The latter is described as a new species on spiders from Ceylon. *G. aranearum* is given as occurring on spiders and possibly on insects (locustids and membracids) throughout most of the world. *Gibellula elegans*, which is placed in synonymy with *G. aranearum*, was described by P. Hennings (**8**) on locusts from Java. It is doubtful that a species on locusts is the same as one on spiders. The identity of *G. phialobasis* on spiders from Java also is doubtful. If the description and illustration given by Penzig and Saccardo (**15**) are correct it is distinct from *G. aranearum*. *Gibellula formosana* Sawada (**23**) has been reported on a moth from Japan but its assignment to the genus *Gibellula* is very doubtful. Kobayasi (**10**) states that it belongs very near *Isaria japonica*. In 1949, the writer (**11**) described *G. formicarum* on an ant from Liberia.

For North America, three species have been reported, *Gibellula aranearum*, *G. arachnophila* and *G. capillaris*. Morgan (**13**) described *G. capillaris* in 1905 from a collection on an insect at Preston, Ohio. His description does not suggest a *Gibellula*. Through the kindness of G. W. Martin, the type specimen, which is in the Herbarium of the University of Iowa, has been seen. It consists of only small fragments from which it was not possible to reach a con-

[1] Paper from the Herbarium and the Department of Botany, University of Michigan. The cost of excess illustrations has been paid by the Herbarium of the University of Michigan.

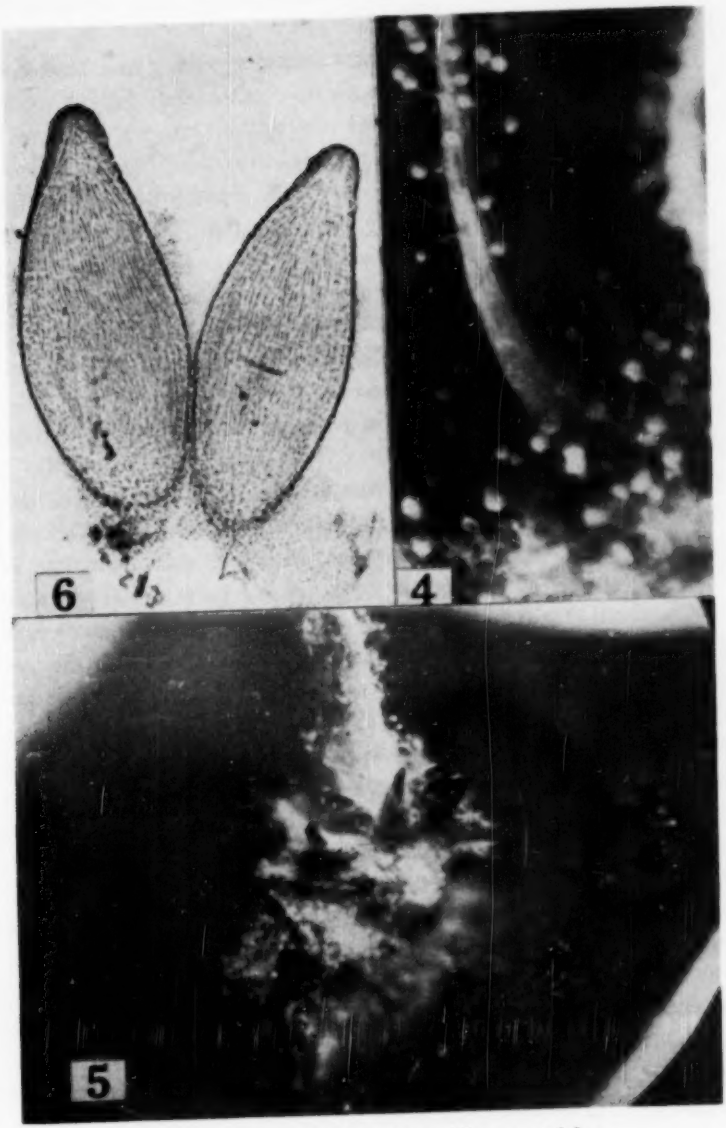


FIGS. 1–3. *Torrubiella arachnophila* var. *pulchra*.

clusion concerning its identity. Both Petch (**18**) and Vera K. Charles (**5**) have decided that the species does not belong in *Gibellula*. It is doubtful that its identity can be established. As shown later the name *G. arachnophila* has been misapplied. According to the treatment by Petch (**18**) one species, *G. aranearum*, is found in North America.

In a study of collections in the Farlow Herbarium,[2] the Herbarium of the New York Botanical Garden, the Mycological Collection of the U. S. Bureau of Plant Industry, and the Herbaria of the University of Michigan and of Cornell University, two species of *Gibellula* have been found to occur on spiders in North America. One is that described by Cavara (**4**) as *Gibellula pulchra* in establishing the genus. It produces a mat of yellow mycelium covering the host and often attaches the host to the substratum. From the mycelium numerous cylindric synnemata (FIG. 1) arise. These consist of more or less parallel, longitudinal, loosely bound hyphae (FIG. 2) and are covered on the outside by a network of brownish, asperulate hyphae which are loosely attached to the synnemata. The conidiophores develop from the outer loose mycelial covering of the synnemata or from the mycelium covering the host (FIGS. 1, 4). They arise from inverted *T*- or *Y*-shaped cells of the outer mycelial covering, one arm of such cells forming the base of the conidiophore (FIG. 2). The conidiophores vary considerably in length and consist of 2–6 cells; all except the terminal cell have brownish, asperulate walls. The terminal cell of the conidiophore has a hyaline, smooth, or very minutely roughened wall. At its point of attachment it is narrower than the cell to which it is attached (FIG. 3). It enlarges above from a slender neck becoming broadly obovoid or ellipsoid in the upper part. The terminal cell bears a number of broadly obovoid prophialides. From the upper portion of each prophialide 6–8 narrowly clavate phialides are produced. From their apices the conidia develop. The cluster of prophialides and phialides form spherical heads, 40–50 μ in diameter (FIG. 3). The conidia apparently are produced in succession from the apices of

[2] The writer is indebted to W. Lawrance White, Donald P. Rogers, John A. Stevenson and Miss K. E. Kindleberger for the loan of specimens for this study.



FIGS. 4–6. *Torrubiella arachnophila* var. *pulchra*.

the phialides. They usually are found attached singly but in some collections adhere in short chains.

The synnemata with the loose mycelial covering and scattered conidiophores have the appearance of a stilbaceous fungus parasitized by an *Aspergillus* or *Sterigmatocystis* (FIG. 1). Saccardo (**22**) described it as *Corethropsis pulchra* attacking an *Isaria*. Cavara (**4**), recognizing its true nature, established the genus *Gibellula* for it and published a good illustration. It also has been well illustrated by Speare (**25**) under the name *G. suffulta*.

Petch has placed *G. pulchra* in synonymy under the name *G. arancarum* (Schw.) Syd., although Sydow (**26**) described *G. arancarum* as a new species without any reference to Schweinitz. *Isaria arancarum* was described by Schweinitz (**24**) in 1822. Fries (**7**) published the species in his Systema Mycologicum with a description evidently taken from Schweinitz. Unfortunately a specimen no longer exists in the Schweinitz Herbarium at the Academy of Natural Sciences of Philadelphia or in the Michener Herbarium, Mycological Collections, Washington. Therefore it is not possible to determine the microscopic characters of the species and establish with certainty its identity. The description given by Schweinitz is not sufficient to definitely establish the application of the name to one of the species of fungi parasitic on spiders.

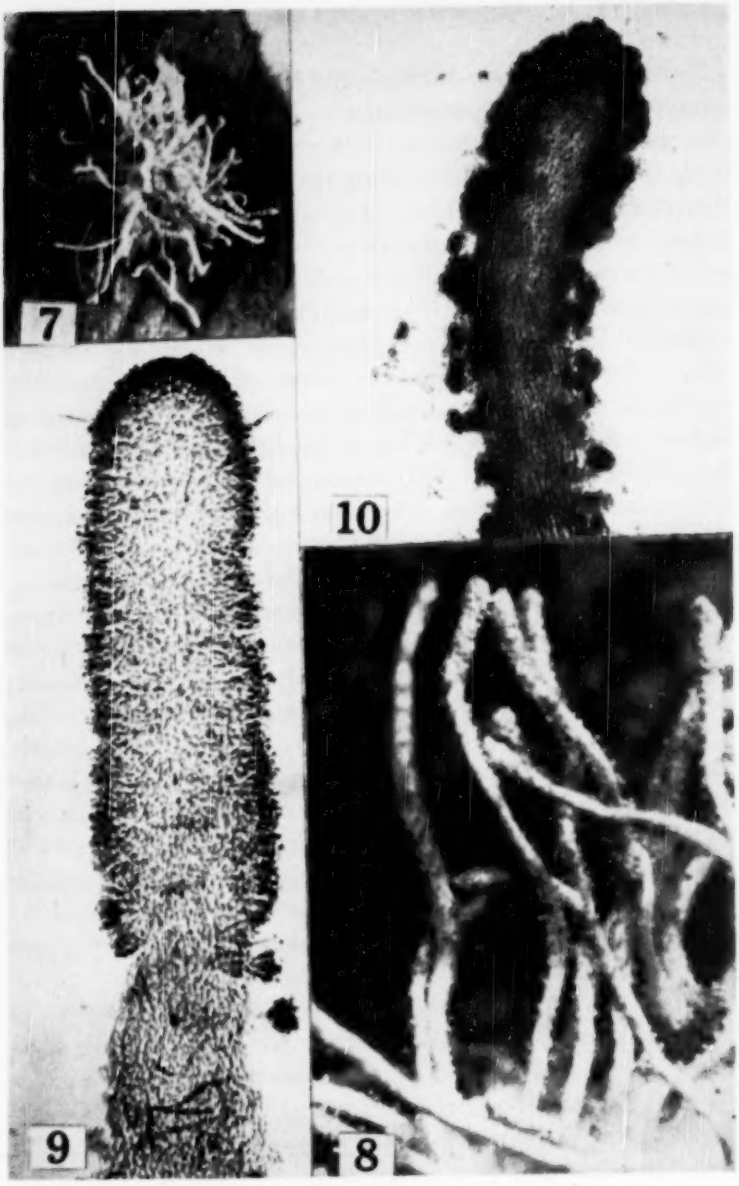
Isaria arachnophila Ditm. on spiders has been reported by several authors who give descriptions and illustrations of a *Gibellula* and Vuillemin (**28**) transferred it to that genus. Fries (**7**) in his Systema Mycologicum included other hosts besides spiders for *I. arachnophila*. However, the name and his reference to Ditmar's description and illustrations indicate that *I. arachnophila* should be applied to a fungus on spiders. It seems evident from Ditmar's (**6**) illustrations that *I. arachnophila* is not a *Gibellula*. It apparently is a *Hymenostilbe* as Petch (**17**) has pointed out.

Wallroth (**29**) described a species on spiders under the name *Isaria tartarea*. His description does not indicate *Gibellula pulchra*, which appears to be the first name which can be applied to the species with certainty. The species has since been described and named *Gibellula suffulta* by Speare (**25**), *G. arachnophila* by

Johnston (**9**), *G. Haygarthii* by Bijl (**1**) and *G. arancarum* by Sydow (**26**).

The other species of *Gibellula* in North America differs from the preceding in several important aspects. It produces conidiophores that are usually much shorter. Often the terminal cell arises directly from the side arm of a cell of the outer mycelial covering of the synnema (FIGS. 11, 12). The length of the conidiophore is usually determined by the length of the side arm of the mycelial cell which may be so short that the terminal cell is practically sessile on the mycelium (FIG. 11) or may be as long as 40 μ (FIG. 12). Occasionally there may be an additional cell between the terminal cell and the mycelium. The broadly obovoid terminal cell of the conidiophore does not have a narrow neck like that of the preceding type but enlarges from a base which is as broad as the cell to which it is attached (FIG. 12). The prophialides are produced from the upper part of the terminal cell and with the phialides form hemispherical to wedge-shaped heads (FIGS. 11, 12), differing thus from the spherical heads of the preceding species. The conidiophores are usually closely crowded, especially over the upper portion of the synnema (FIGS. 9, 10). The almost sessile heads give a tufted appearance to the synnemata which even under low magnifications makes this species easy to distinguish from the preceding (compare FIGS. 7 and 8 with 1 and 4). Petch (**18**) apparently discusses these species as variations of *G. arancarum*. He places most emphasis on length of the conidiophores and states that in some collections of the second type there is a transition from stalked to sessile heads on the same synnema. In such specimens seen by the writer the shapes of the terminal cells and heads of the conidiophores are distinctive. These distinctions justify the recognition of two species.

The second species has been described and illustrated by Oudemans (**14**) and Boudier (**3**) under the name *Isaria arachnophila* Ditm. Vuillemin (**28**) also has discussed and illustrated it under the name *Gibellula arachnophila* (Ditm.) Vuill. giving *Isaria arachnophila* Ditm. as a synonym. Petch (**18**) also concluded that Wallroth (**30**) described this species under the name *I. arachnophila* Ditm., "tegmen tartareum" referring to the tufted condition of the



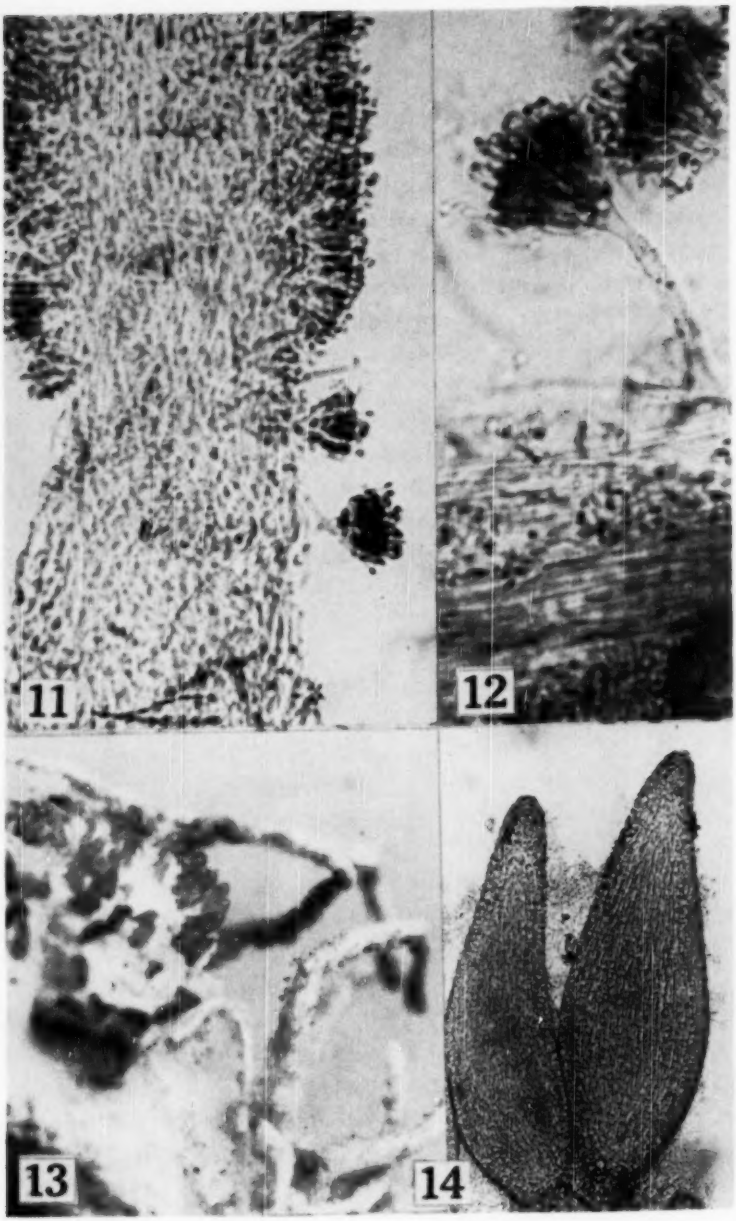
FIGS. 7–10. *Torrubiella arachnophila* var. *pleiopus*.

synnemata. As has already been stated *I. arachnophila* Ditm. is doubtless a *Hymenostilbe* and the name therefore has been misapplied to a *Gibellula*.

In 1920 Maublanc (**12**) proposed two forms of *G. arachnophila*, forma *macropus* Vuill. and forma *leiopus* Vuill. Although they were distinguished mostly on the lengths of the conidiophores the illustrations of Maublanc and Vuillemin (**28**) indicate without much question that forma *macropus* is *G. pulchra* and forma *leiopus* is the second species under discussion. As already stated these are separated by other important differences. Therefore the name **Gibellula leiopus** (Vuill.) comb. nov. is proposed for the second species.

Among the North American collections a few specimens of both *G. pulchra* and *G. leiopus* bear perithecia. In the perithecial stage they show no important differences. The perithecia develop in the mycelium covering the host and are at first embedded except for their apices. The shrinkage of the mycelium in herbarium specimens leaves the perithecia only partly embedded but usually with a thin covering of hyphae on the lower portions (FIGS. 5, 6, 13, 14). The asci are long and narrow, containing eight filiform multiseptate ascospores. The perithecial stages of these fungi place them in the genus *Torrubiella*.

Under the name *Cordyceps arachnophila*, Johnston (**9**) in 1915 described perithecia associated with synnemata of a *Gibellula* on spiders from Cuba. His description is that of a *Torrubiella*. Johnston implies that the species had previously been described since he makes the following statement. "The perfect stage of a Gibellula has at least in one case been described as a Cordyceps. What appears to be this species has been studied by the writer in Cuba." He does not give a reference to the place of publication or the authority for the species. Apparently the only previous use of the name was by Thaxter (**27**) in 1914. In a paper concerning the genus *Aschersonia* he states that the perithecial stages of some species of *Aschersonia* "might at first be mistaken for the common *Cordyceps* (*Torrubiella*) *arachnophila*, which is often found on leaves with or without its imperfect or *Isaria* (*Gibellula*) condition." A description is not given and the name therefore was not validly



FIGS. 11–14. *Torrubiella arachnophila* var. *pleiopus*.

published. As far as the writer has been able to determine Johnston's publication of *Cordyceps arachnophila* is the first description of perithecia associated with a *Gibellula* on spiders. Since the fungus is a *Torrubiella* the combination *Torubiella arachnophila* is proposed. Johnston describes the conidial stage under the name *Gibellula* (*Isaria*) *arachnophila* without a reference to Ditmar or to other authorities. His description indicates that it is *Gibellula pulchra*. All the collections cited are from Puerto Rico. It is not certain whether the conidial stage accompanying the perithecia of the Cuban material is the same.

Petch (**18**) in 1932 reported perithecia of a *Torrubiella* occurring in four collections having conidiophores of "*Gibellula aranearum*" and has described the perithecial stage under the name *Torrubiella gibellulae* Petch. In these collections synnemata were lacking, the conidiophores occurring on the mycelium with the perithecia. Petch does not describe the conidiophores which accompany the perithecia. *Torrubiella gibellulae* appears to be the same as *T. arachnophila*.

In general the species of *Torrubiella* on spiders show less marked differences in their perithecial stages than in the conidial. The description of *I. flava* Petch on spiders does not indicate important differences from *I. arachnophila*. This conclusion is supported by specimens of the two species determined by Petch in the Farlow Herbarium. However, Petch (**16**) states that the conidial stage of *I. flava* is a *Hirsutella* and therefore it is very distinct in its conidial stage. Several other species of *Torrubiella* on spiders also show much greater differences in their conidial stages than in the perithecial. *Torrubiella aranicida* Boud., the type of the genus, is reported by Boudier (**2**) as having *Isaria cuneispora* Boud. as its conidial stage. Petch (**19**) states that *Cephalosporium aranearum* Petch is the conidial stage of *Torrubiella alba* Petch, *Spicaria longipes* Petch (**20**) that of *T. gonylepticida* (Möller) Petch and *Cylindrophora aranearum* Petch (**21**) that of *T. albolanata* Petch. It is not possible at the present to determine specific limitation and relationships of the species of *Torrubiella*. This must await a restudy of the genus as a whole. It does not seem advisable therefore to propose additional species of *Torrubiella* on spiders on the differences which have been noted. These differences, however,

are sufficient to justify the recognition of two varieties of *Torrubiella arachnophila* distinguished by their conidial stages, and consequently *Torrubiella arachnophila* var. *pulchra* with *Gibellula pulchra* Cavara as its conidial stage and *T. arachnophila* var. *leiopus* with *G. leiopus* (Vuill.) Mains as its conidial stage are proposed.

In the following descriptions (FH) indicates that the specimen is in the Farlow Herbarium, Harvard University, (NY) in the Herbarium of the New York Botanical Garden, (W) in the Mycological Collections Bureau of Plant Industry, Washington, (M) in the Herbarium of the University of Michigan and (C) in the Herbarium of the Department of Plant Pathology of Cornell University.

Torrubiella arachnophila (Johnston) comb. nov.

Cordyceps arachnophila Johnston, Bul. Porto Rico Insular Exp. Sta. **10**: 23. 1915.

Torrubiella gibellulae Petch, Ann. Mycol. **30**: 391. 1932.

Perithecia arising from a yellow to yellowish white mat of mycelium covering the host, scattered or crowded in small groups, at first embedded in the mycelial covering except for the apices, becoming more or less superficial, the lower portions covered with a thin layer of hyphae, light yellowish brown to reddish brown above, narrowly ovoid to conoid, 550–1200 × 230–350 μ; asci narrowly cylindric, 450–660 × 4–6 μ, the wall thin, thickened into a cap at the apex, 4–5 μ thick; ascospores filiform, hyaline, nearly as long as the asci, 1.5 μ wide, multiseptate, tardily breaking into one-celled fragments, the cells 4–10 μ long.

Torrubiella arachnophila var. **pulchra** var. nov. (FIGS. 1–6)

Status conidicus *Gibellula pulchra* (Sacc.) Cavara, conidiophoris 2–4 septatis, 150–600 μ longis, cellis terminalis attenuatis deorsum late ellipsoideis vel obovoideis sursum, gerentibus capitula spherica. Perithecia ovoidea, 900–1200 × 300 μ, ascis cylindricis, 600–660 × 5–7 μ, ascosporis filiformibus, multiseptatis. Specimen typicum in araneis, Burbank, Tenn., R. Thaxter, 4122, Aug. 1896, in Herb. Mich.

The distinctive conidial stage of the variety is *Gibellula pulchra* for which the following description is given. All the collections listed have the conidial stage. The one collection with perithecia is noted.

GIBELLULA PULCHRA (Sacc.) Cavara (FIGS. 1–4)

Att. Instit. Bot. Univ. Pavia Ser. II, **3**: 347. 1894.

Corethropsis pulchra Sacc., Michelia **1**: 83. 1877.

Corethropsis australis Speg., An. Soc. Cient. Arg. **13**: 24. 1882.

Gibellula suffulta Speare, Phytopath. **2**: 137. 1912.

Gibellula arachnophila Johnston, Bul. Porto Rico Insular Exp. Sta. **10**: 24. 1915. Not *Gibellula arachnophila* (Ditm.) Vuill. 1911.

Gibellula arachnophila f. *macropus* Vuill. apud Maublanc, Bul. Soc. Mycol. France **36**: 41. 1920.

Gibellula Haygarthii Bijl, Trans. Roy. Soc. S. Africa **10**: 149. 1922.

Gibellula aranearum Syd., Engler's Bot. Jahrb. **62**: 321. 1922.

Synnemata arising from a yellow or yellowish mat of mycelium covering the host, cylindric, yellowish brown to violaceous brown when dried, more violaceous when fresh, sometimes slightly enlarged above, 3–9 mm. long, 100–300 μ thick below, 200–400 μ thick above, consisting of multiseptate somewhat loosely bound longitudinal hyphae; conidiophores arising from the mycelium covering the host or from a network of brownish asperulate hyphae loosely attached to the surface of the synnemata, 150–600 μ long, 7–12 μ wide, the lower 3–5 cells brownish, asperulate, the terminal cell hyaline, smooth, 2–3.5 μ thick below, enlarging from a slender neck into a broadly ellipsoid or obovoid head, 6.4–10 μ wide bearing up to 12 broadly obovoid prophialides measuring 6–12 $\times$ 4–6 μ, each prophialide in turn bearing at the apex clusters of 6–8 narrowly clavate phialides measuring 6–10 $\times$ 2–3 μ, the prophialides and phialides forming spherical heads 30–42 μ in diameter; conidia fusoid to fusoid-ellipsoid, 2.5–6.4 $\times$ 1.5–2.5 μ, produced in succession from the apices of the phialides, occurring singly or in short chains.

On spiders.

Delaware: Noaman's Creek, Brandywine Hd., Aug. 16, 1892, A. Commons (NY).

Maine: York, Aug. 1895, R. Thaxter (FH *6247*), Sept. 1895 (FH *6239*); Millinocket, 8-23-40, Steinmetz (C *29183*).

Massachusetts: Waltham, Sept. 1886, R. Thaxter (FH *6268*); Canton, Aug. 1936, A. M. & D. P. Rogers *2091* (NY).

North Carolina: Cranberry, 1887, R. Thaxter (FH *6253*).

Tennessee: Burbank, Aug. 1896, R. Thaxter, perithecia, type (M and FH

4122); Cades Cove, Great Smoky Mt. Nat. Park, Aug. 20, 1939, D. H. Linder (FH).

Puerto Rico: Rio Piedras, Aug. 31, 1913, J. R. Johnston (FH *6283*); Rio Piedras, Dec. 27, 1913, J. R. Johnston (FH *6282*); San Juan, March 2, 1923, G. N. Walcott (W); Arecibo, Faxon, Mills & Anderson *2940* (W); Barceloneta, Nov. 20, 1934, C. G. Anderson & A. S. Mills (W); Pueblo Viejo, Faxon, Anderson and Mills (W).

The following collections from outside of North America have been seen.

Venezuela: Margarita, June 1948, Blakeslee (FH *754*); Aragua, April 5, 1938, Carlos E. Chardon, H. H. Whetzel, A. S. Müller *2786* (C); Central Lucinda, Yaracuy, Carabobo, Jan. 8, 1938, Carlos E. Chardon *2444* (C); Rancho Grande, Aragua, March 28, 1939, E. Chardon, H. H. Whetzel, A. S. Müller *3235* (C).

British Guiana: C. B. Williams, Aug. 28, 1916 (FH *748*).

Trinidad: 1912–13, R. Thaxter (FH *2572*); Maraval Valley, 1912–13, R. Thaxter (FH *2574*); Port-of-Spain, 1912–13, R. Thaxter (FH *6222B, 6223*); Maraval Reservoir, Port-of-Spain, Feb. 8, 1913, R. Thaxter (FH *6124*); La Seiva Valley, May 6, 1913, R. Thaxter (FH *2564*); St. Anne Valley, June 1913, R. Thaxter (FH *2571*); Feb. 1917, J. B. Rorer (FH *639, 2566*); Port-of-Spain, J. B. Rorer (FH *2585*).

Chile: Corral, 1905–06, R. Thaxter (FH *6119B*).

Territory Hawaii: Kokee, March 9, 1898, C. L. Shear (W).

New Guinea: Morobe, Nov. 11, 1939, Mrs. Clemens (W).

Torrubiella arachnophila var. **pleiopus** var. nov. (FIGS. 7–14)

Status conidicus, *Gibellula pleiopus* (Vuill.) Mains, conidiophoris 1–2-septatis, brevibus, cellis terminalibus late obovoideis vel obpyriformibus non attenuatis deorsum, gerentibus capitula hemisphericia, cuneiformia vel obconica. Perithecia ovoidea, 550–900 × 230–350 μ, ascis cylindricis, 400–600 × 5–6 μ, ascosporis filiformibus, multiseptatis. Specimen typicum, Newfane Hill, Vermont, Aug. 1940, V. K. Charles in Herb. Mich.

The distinctive conidial stage of the variety is *Gibellula pleiopus* for which the following description is given. All the collections listed have the conidial stage. The few with perithecia are so noted.

GIBELLULA PLEIOPUS (Vuill.) Mains (FIGS. 7–12)

Gibellula arachnophila f. *pleiopus* Vuill. apud Maublanc Bul. Soc. Mycol. France **36**: 42. 1920.

Synnemata arising from a yellow to whitish mat of mycelium covering the host, cylindric, 1.5–8 mm. long, 80–300 μ thick, consisting of multiseptate longitudinal hyphae somewhat loosely bound together, light grayish brown to violaceous brown when dried, often reported as violaceous when fresh, the surface having a tufted ap-

pearance; conidiophores very short, often scattered on the lower part of the synnemata, usually crowded on the upper part, especially over the apices, arising from a network of brownish, asperulate hyphae loosely attached to the surface of the synnemata, usually the lower portion of a conidiophore being a lateral arm of a cell of the hyphal net, the terminal cells usually produced directly on the side arms, occasionally having an intermediate cell, the lower portion of the conidiophore brownish and asperulate, the terminal cells hyaline and smooth below as wide as the hypha to which it is attached, enlarging upward, broadly obovoid or obpyriform, 10–18 × 4.5–8 μ, bearing a number of prophialides on the upper portion, the prophialides broadly obovoid to ellipsoid, 7.5–12 × 3–5 μ, bearing up to 4 phialides at their apices, the phialides narrowly clavate to subcylindric, 7.5–12 × 2.5–3.5 μ, the walls thin below, slightly thickened at the apices, the prophialides and phialides together forming wedge-shaped to broadly obconic heads, 30–50 μ across; conidia fusoid to fusoid-ellipsoid, 3–8 × 1–2 μ, produced successively from the apices of the phialides, occurring singly or in short chains.

On spiders.

Maine: Kittery Point, Aug. 1886, R. Thaxter (FH *6260*); Kittery Point, July 1886, R. Thaxter (FH *6270*); Kittery Point, Sept. 1891, R. Thaxter (FH *650*); Kittery Point, 1895, R. Thaxter (FH *6251*); Kittery Point, R. Thaxter (FH *6206*).

Maryland: Newfield, Oct. 10, 1875, Ellis Coll. (NY).

Massachusetts: Woods Hole, Sept. 22, 1881, Trelease (FH); Waltham, Sept. 1886, R. Thaxter (FH *6272*); Canton, July 11, 1936, D. H. Linder (FH); Hamilton, July 18, 1937, D. L. Vickar (FH); Harvard Forest, Petersham, Aug. 19, 1949, C. Alexopoulos and C. T. Rogerson *3186* (M).

New Hampshire: Chocorua, Aug. 1904 (FH); Chocorua, Sept. 1906, W. G. Farlow (FH); Chocorua, Aug. 4, 1907 (FH); Shelbourn, Aug. 8, 1940, V. K. Charles (W).

New York: Coy Glen, Ithaca, Aug. 22, 1935, J. A. Stevenson (W).

North Carolina: Cranberry, 1886–1887, R. Thaxter (FH *6159*); Cranberry, Aug. 1887, R. Thaxter (FH *755*, *6263*); Cranberry, 1887, R. Thaxter (FH *6261A*); Cranberry, Aug. 2, 1896, R. Thaxter, perithecia (FH *6169*).

New Jersey: Newfield, March 29, 1896, Ellis Coll. (NY).

Vermont: Newfane Hill, Aug. 1939, V. K. Charles, perithecia, several collections (M, W); Newfane Hill, Aug. 1940, V. K. Charles, perithecia, type (M, W); Newfane Hill, Aug. 1941, V. K. Charles, several collections (W).

Virginia: Great Falls, June 11, 1919, C. H. Kauffman (M); Hoover Camp, Nov. 1, 1935, J. A. Stevenson (W); Mountain Lake, Sept. 4, 1936, A. E. Jenkins (W); Mountain Lake, Sept. 4, 1936, J. A. Stevenson (W); Limberlost, Sept. 9, 1937, V. K. Charles (W); Sept. 9, 1937, J. A. Stevenson (W).

Wisconsin: Madison, 1882, Trelease (FH); Madison, Nov. 19, 1884, Wm. Trelease (FH *6420*).

Nova Scotia: Brookside, July 13, 1931, L. E. Wehmeyer *1015, 1015A* (M).

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EXPLANATION OF FIGURES

FIGS. 1–6. *Torrubiella arachnophila* var. *pulchra.* FIGS. 1–4, the conidial stage, *Gibellula pulchra;* 5, 6, the perithecial stage. FIG. 1. Synnemata arising from the host showing scattered conidiophores, × 15. FIG. 2. Conidiophores arising from the outer loose mycelium of a synnema, × 280. FIG. 3. Upper portion of a conidiophore showing asperulate wall of a lower cell, narrow neck of the terminal cell and spherical cluster of prophialides, phialides and conidia, × 700. FIG. 4. Showing conidiophores arising from the mycelium as well as from a synnema, × 150. FIG. 5. Perithecia partly embedded in the mycelium covering the host, × 15. FIG. 6. Perithecia, × 80.

FIGS. 7–14. *Torrubiella arachnophila* var. *pleiopus.* FIGS. 7–12, the conidial stage, *Gibellula pleiopus;* 13, 14, the perithecial stage. FIG. 7. Synnemata arising from the host, × 3. FIG. 8. Synnemata showing tufted surface, × 15. FIG. 9. Short synnema showing the short conidiophores in a compact layer, × 100. FIG. 10. Upper portion of a synnema showing tufted conidiophores, × 100. FIG. 11. Showing conidiophores consisting of a wedge-shaped head of prophialides and phialides borne on sessile terminal cells, × 200. FIG. 12. Conidiophore consisting of a short asperulate cell, obovoid terminal cell and wedge-shaped head of prophialides and phialides, × 500. FIG. 13. Perithecia partly embedded in the mycelium covering the host, note associated synnemata, × 15. FIG. 14. Perithecia, × 80.

A NOTE ON NAUCORIA MYOSOTIS

ALEXANDER H. SMITH [1]

(WITH 1 FIGURE)

This striking species of our northern regions and bogs was transferred to *Flammula* by Singer (Rev. de Myc. **2**: 241. 1937) and placed in the subgenus Phaeo-Naematoloma of *Flammula*. Favrè has given a detailed account of the fungus (Bull. Soc. Myc. Fr. **52**: 143. 1936). In setting up his subgenus Singer was impressed by the resemblance of the fungi he placed here to the slender species of *Naematoloma* such as *N. udum*, *N. ericeum*, etc. He made no mention of the characteristic naematolomoid pleurocystidia in his diagnosis of the subgenus. Presumably, the members of this group did not possess them and that, of course, would be a good reason for excluding the species from *Naematoloma*.

In connection with a work which is in progress on *Galerina*, North American collections of *N. Myosotis* were examined to check the possibility of placing the species in that genus. All our collections were found to have the characteristic pleurocystidia of the genus *Naematoloma*. Since Favrè made no mention of such organs in his account of the species, an examination of specimens kindly sent us by Favrè was made. Pleurocystidia of the *Naematoloma* type were found; in fact from our study it was clear that no significant differences between the American and European specimens existed.

Smith (Mycologia **40**: 670. 1948) pointed out previously that if the group of genera *Stropharia*, *Naematoloma* and *Psilocybe* were to be established on a natural as opposed to an artificial basis, spore color in deposits would have to be given less emphasis than was given it in the Friesian classification, *i.e.*, some species with dark brown spore deposits, but with all the anatomical and morphological features of these genera, would have to be admitted to them.

[1] Papers from the Herbarium and the Department of Botany, University of Michigan, Ann Arbor, Michigan.

Naucoria Myosotis is a good representative of this group and is hereby transferred to *Naematoloma*.

Naematoloma Myosotis (Fr.) comb. nov. (= *Agaricus Myosotis* Fr. Syst. Myc. **1**: 290. 1821)

Pileus (10) 15–30 (40) mm. broad, broadly conic to convex, glabrous except occasionally with scattered veil remnants along the margin, edge often appendiculate, viscid to glutinous, evenly "medal bronze" to "citrine" (olivaceous or greener), evenly colored, opaque when young but before fading becoming brownish and striatulate, near olive buff faded; flesh dull greenish, pliant, odor and taste not

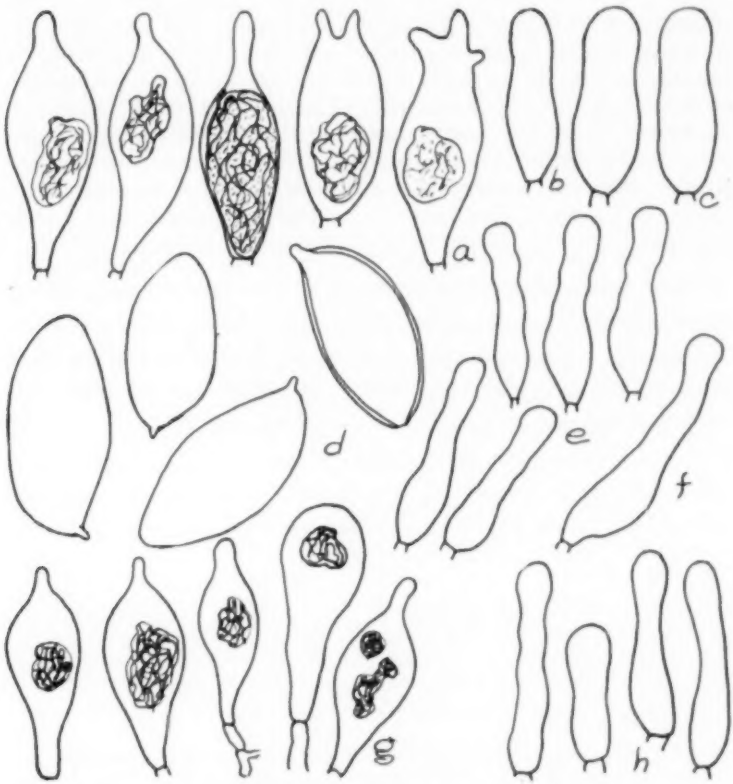

FIG. 1. Spores and cystidia of *N. Myosotis:* a, b, c, d, e, f—from sm–33–988. g and h—from a collection by Favrè.

distinctive; lamellae adnate to shallowly adnexed, equal to slightly ventricose, subdistant, broad, whitish at first, then pale olivaceous and finally brown from the spores, edges minutely white fimbriate and eroded; stipe (6) 10–15 (20) cm. long, 2–5 mm. thick, very rigid, equal, hollow, covered to line formed by the broken veil by white to olivaceous veil fibrils in patches or zones, densely pruinose above.

Spores 14–17 × 7–9 μ, narrowly inequilateral in side view, narrowly ovate in face view, ochraceous tawny in KOH and smooth to very slightly wrinkled (as revived in KOH), the wall relatively thick, apical pore present but apex not truncate; basidia four-spored, projecting when sporulating, hyaline in KOH, 26–34 × 9–11 μ; pleurocystidia present, 35–46 × 10–13 μ, ventricose-mucronate or with several protuberances over upper portion, some ventricose with broadly rounded apices, hyaline and homogeneous in water mounts of fresh material but with a highly refractive amorphous content variously disposed as revived in KOH; cheilocystidia abundant, 32–46 × (6) 8–12 μ, the broadest cells usually with highly refractive content, narrowly ventricose and apices obtuse, thin-walled; gill trama subparallel, olivaceous to yellow in KOH; pileus trama with a well differentiated gelatinous pellicle of hyphae 3–5 μ diam., cells beneath much larger and yellow in KOH (content olivaceous fresh), clamp connections present.

Habit, habitat and distribution: Gregarious on wet humus along edges of bogs, late summer and fall. It is typically northern in its distribution. The description is from Smith 33-988, Huron Mts., Sept. 16, 1933, Mains and Smith. The drawings are from this collection and one by Favrè from the Alps.

NOTES AND BRIEF ARTICLES

ARTICLE 57

CHARLES GARDNER SHAW [1]

Article 57 has been cited so many times in discussions of nomenclatorial problems that most mycologists interested in such matters should be able to quote it *verbatim*. Nevertheless, the first two paragraphs of the article are reprinted once again.

Art. 57. Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema*, or Persoon, *Synopsis*, to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the Rules. The perfect state is that which ends in the ascus stage in the *Ascomycetes*, in the basidium in the *Basidiomycetes*, in the teleutospore or its equivalent in the *Uredinales*, and in the spore in the *Ustilaginales*.

Generic and specific names given to other states have only a temporary value. They cannot replace a generic name already existing and applying to one or more species, any one of which contains the "perfect" form (**2, 3**).

It has been stated recently (in a discussion of *Milesina* vs. *Milesia*) that a name "given" with a description of an imperfect state of a fungus is also "given" to the perfect state of the same fungus, even though no mention of the perfect state is made, if reexamination proves that the perfect state is actually present in the type material (**4**, p. 250–251). The argument is carried further in a discussion concerning *Albugo* and *Cystopus* (**5**, p. 432–433).

The applicability of *Albugo* and of *Cystopus* Lév. to the perfect state depends not on whether the authors who published them recognized the presence of oospores, but on whether oospores were present in their specimens. To argue otherwise would be to discard, as names applied to the imperfect state, all names applied to Basidiomycetes without recognition of

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the presence of basidia—i.e., most names published before 1837, and many later ones. It seems reasonable to suppose that at least a part of the material that Persoon (or Gray) and Léveille had in hand contained oospores, even though the authors did not describe them. It is therefore submitted that their names are not rendered "of only temporary value" by Article 57 (**5**).

I cannot agree that a name has been "given" to a state which the author of that name did not mention in any way in his description. Nor do I believe that this doctrine necessitates abandoning most early names for Ascomycetes and Basidiomycetes.

A name published without a description is a *Nomen nudum* (Art. 37). A name published on printed labels attached to herbarium packets and distributed with the specimens does not constitute valid publication of that name (Art. 37), unless there is also a description (or reference to a previously published description), in spite of the fact that the specimen contained in the packet may be the best material that any past, present, or future mycologist might wish. The name printed and published on the packet in which that specimen is contained is a *Nomen nudum*, in spite of the fact that the name has a type. In other words, article 37 takes precedence over article 18. Stated another way the binomial on the packet is not "in conformity with (all) the Rules" (Art. 57).

It is my contention that article 57 as now worded makes *Nomina provisiora* of all binomials "given" to anything other than the perfect states of pleomorphic fungi. In this connection the following excerpt of article 57 is important: "The earliest (name) . . . given . . . to the state containing . . . the perfect form, *provided that the name is otherwise in conformity with the Rules.*" I interpret this to mean that article 37 (as well as all the rest of the Rules) must be complied with. Therefore, the name "given" to the perfect state must be a validly published name *for that state, i.e.*, it must be "accompanied by a description . . . or by a reference to a previously and effectively published description" of that state (Art. 37). (The wording of article 57 would certainly be improved if "given . . . to" were changed to "validly published for.")

If descriptions of the perfect state are necessary to validate names among the Oomycetes and Uredinales, then they are also required

for the perfect states of other groups (*e.g.*, Ascomycetes and Basidiomycetes). Rogers, then, sees a possibility that many of the earlier generic names applied to Basidiomycetes may be invalid (**4**, **5**). This may be the main reason for his arguments against the necessity of a description of the perfect state.

Any attempt to circumvent the requirement of a published description for the stage on which the nomenclature of an organism is based must be resisted.

The size of any fungus collection is limited; the number of cotypes into which the original collection can be divided is usually small and consequently distribution of the type material is limited. Often the original collection is sufficient for but one specimen which is deposited in a single herbarium. Neglect, the ravages of time, repeated examination of the type by an ever increasing number of mycologists, or other acts of man could destroy all of the type material of a given species.

The printed word is much more permanent. Once a publication is distributed, neither a single catastrophe nor even several simultaneous catastrophes are likely to destroy all copies of a given publication. Publications are man made. When the reservoir of a publication becomes depleted, it can be reproduced (as has been done with Saccardo's "Sylloge" and as has been proposed for certain works of Persoon, Fries, Sydow, etc.). Therefore publications have permanency.

The material contained in a type collection is not man made and cannot be reproduced by man or otherwise exactly duplicated when it is lost or destroyed. It is an unassailable fact that the last sentence of article 18 now applies to many species; it is my contention that time only will determine the date when it must be applied to all but the most recently discovered species. "Where permanent preservation of a specimen or preparation is impossible, the application of the name of a species . . . is determined by means of the original description or figure" (Art. 18).

A description then has greater permanency, not to mention greater accessibility, than the original specimen. Furthermore a description of the perfect state must be "given" (*i.e.*, validly published) if article 57 and the rest of the Rules are complied with.

The law on this point is *not* contained in article 18 (**4**, p. 251), but rather in the Rules as a whole.

Even so article 57 does not necessitate abandoning a great many of the earlier names given to Basidiomycetes and Ascomycetes. Let us determine what the perfect state is. Rogers has gone into this thoroughly and concludes "the one meaning of 'state' that makes sense with the whole text of the Rule and with all the examples is 'fructification'" (**4**, p. 246). With this I agree. The second sentence of article 57 then reads: "The perfect *fructification* is that which *ends* in the ascus state in the *Ascomycetes*, in the basidium in the *Basidiomycetes*, in the teleutospore or its equivalent in the *Uredinales*, and in the spore in the *Ustilaginales*." Note it is not stated that the perfect fructification *is* the ascus, the basidium, the teleutospore, or the spore respectively, but merely "that which ends" in these structures, *i.e.*, the ascocarp, the basidiocarp, the telium, and the smut sorus.

Since previous discussions concerning this point have used the Oomycetes, the Uredinales, and the higher Basidiomycetes as examples, the same examples are used here. What is the perfect fructification (state) in the Oomycetes? It is the oogonium and the oospore(s) contained therein (and possibly also the antheridium). There are no surrounding sterile fungous structures making up a part of the fructification. The oogonia (containing oospores) are scattered throughout the cortical tissues of the host; there may be a great many or a very few in a given specimen, but there is no organization into a more complex structure. Thus, the perfect fructification in the Oomycetes is the oogonium and its contents, the oospore (or oospores). In order to comply with article 57 and the rest of the Rules, the oogonium and its contents must be described. I have indicated elsewhere that article 57 as now worded does apply to the Phycomycetes (**6**).

In the Uredinales the perfect fructification is the telium or teleutosorus ("that which ends . . . in the teleutospore or its equivalent"). The telium is essentially a mass of teliospores, although in some species (*e.g.*, *Puccinia allii*) paraphyses are present, and in some microcyclic rusts (*e.g.*, *Endophyllum sempervivi*) a peridium is present (**1**). The telium (the perfect fructification) then,

whatever its shape, structure, and component parts, is the structure which must be described if article 57 is complied with. (Rogers (**4**, p. 241) has pointed out that in the authoritative French text of the Rules (**3**) *Uredo* is considered an imperfect state.) It seems doubtful that a telium can be described adequately without mentioning its primary, and frequently only, morphologically significant component, the individual teliospores.

In the Homobasidiomycetes the perfect fructification (state) is a much more complex structure than that of either the Oomycetes or the Uredinales. The basidiocarp is usually composed of several distinct tissues and many different structures of which the basidium is but one. Furthermore, here, to a much greater extent than in the two previously cited groups, the perfect fructification exhibits many macroscopic characters which can be described and used as taxonomic criteria. The basidiocarp (the perfect fructification) then is the structure which article 57 requires be described; article 57 does not require that the basidium itself be described. The situation in regard to the Ascomycetes is similar to that in the Basidiomycetes; the ascocarp must be described, not necessarily the ascus.

The comparable perfect fructifications in the groups discussed are the oogonium and its component parts (few in number), the ascocarp and its component parts, the telium and its components (again few in number), and the basidiocarp with its component parts. The oospore, the ascus, the teliospore, and the basidium are not fructifications, but in each case merely a component part of a perfect fructification.

If the reasoning presented above is accepted, it becomes necessary to conserve *Cystopus* de Bary vs. *Cystopus* Blume (**4**). It is so proposed. Concerning *Milesina* Magnus vs. *Milesia* F. B. White no conservation is necessary since *Milesina* conforms with the Rules, while *Milesia* as published was a *Nomen provisiorum*.

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CONCERNING THE CONSERVATION OF THE NAME RHODOPHYLLUS

Rogers (Farlowia **3**: 474–5) is not in favor of conserving this name against *Acurtis, Entoloma,* etc., on the grounds that it is superfluous, and he doubts that conserving it would stabilize nomenclature or avoid disadvantageous changes. He rejects the name *Acurtis* as being based on an "imperfect state." The genus *Acurtis* was based on the carpophoroid stage of *Rhodophyllus abortivus*. Rogers apparently assumes that the "perfect state" in Basidiomycetes is the basidium. I have a letter from Singer dated Jan. 31, 1940, which he states that "Acurtis *does* form basidiospores in some individuals." This evidence, is sufficient to vitiate Rogers' reason for rejecting *Acurtis*.

However, I am not willing to accept the thesis that the basidium itself is the perfect state in the gill fungi. At present the rules read "The perfect state is that which ends . . . in the basidium in the Basidiomycetes. . . ." This clearly applies to the *fruiting stage* in the gill fungi. As far as this group of fungi is concerned nothing could be clearer, and any proposed changes in article 57 which might confuse the situation in this group should not be accepted by the coming Congress. Let us consider what a failure to conserve *Rhodophyllus* will mean:

1. For those who believe, as I do, in the current broad concept of *Rhodophyllus*, all pink-spored agarics with angular spores will have to be transferred to *Acurtis*. This will mean several hundred new combinations.
2. Those who are willing to disregard the name *Acurtis*, but who still use the broad concept of the genus will be forced to

use *Entoloma* and if so, only about two hundred new transfers will be necessary.

3. Those who wish to maintain most of the Friesian groups intact as genera, but who believe that species with smooth longitudinally striated spores (*Clitopilus*) are generically distinct from the angular spored species with decurrent gills (*Acurtis*), will have to use *Acurtis* if they follow the rules and thus introduce *another* generic name, and one which has never been used, in the gill fungi. To me this is clearly a disadvantageous change.

It seems to me that all of Rogers' reasons for voting against the conservation of the name *Rhodophyllus* have been effectively answered. Further discussion would merely belabor the question. In summary, however, it might not be out of order to mention the advantages to be gained by conserving the name.

1. Those who use the broad concept of the genus have an admirably descriptive name which is now in use by the majority of specialists in the gill fungi.
2. Most of the transfers have already been made so definite stability of names will be attained.
3. It will not be necessary to introduce a previously unused generic name for any of the fungi involved.

It is admittedly most unfortunate that Quélet did not use one of his previously established names when he set up his concept of *Rhodophyllus*. But since the name has gained wide acceptance, in this case it is best to keep it. In the question of *Drosophila* vs. *Psathyrella* the opposite is true since both are in use by specialists, and the proponents of *Drosophila* cannot present a case for its conservation which is parallel to that of *Rhodophyllus*.—ALEXANDER H. SMITH.

CONCERNING THE AUTHORSHIP OF MARSSONIA

The first fungus collection referred to the genus *Marssonia* appeared in Rabenhorst's Fungi europaei exsiccati, no. 1857

(1874) as *Coleroa potentillae* (Wallr.) Fr. with the collection note "leg. Fischer (sub nom. '*Marssonia potentillae* Fisch.')." The botanist Fischer's given name has never been revealed anywhere in the extensive literature concerning *Marssonia*. Although Magnus (Hedwigia **45**: 88–91. 1906.) later substituted the name *Marssonina* for Fischer's *Marssonia*, the identity of Fischer should be of historical interest. After a series of articles by Arthur (Phytopath. **23**: 559–561; 841. 1933.) and Spalding (Phytopath. **23**: 203–204. 1933.) concerning the authorship of the rust species *Cronartium ribicola*, H. Sydow (Ann. Myc. **32**: 115–117. 1934.) established the author to be Johann Carl (Karl) Fischer, a botanist of Stralsund, Pomerania. The specimens of both *C. ribicola* and *M. potentillae* were collected and named by Fischer at Stralsund and distributed in Rabenhorst's Fungi europaei exsiccati as nos. 1595 and 1857, respectively. In addition, Magnus (1906) depicted the author of *Marssonia* as "der Stralsunder Lehrer Fischer." These and related facts leave no doubt that the author of *Marssonia* was Johann Carl Fischer.—PAUL L. LENTZ.

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